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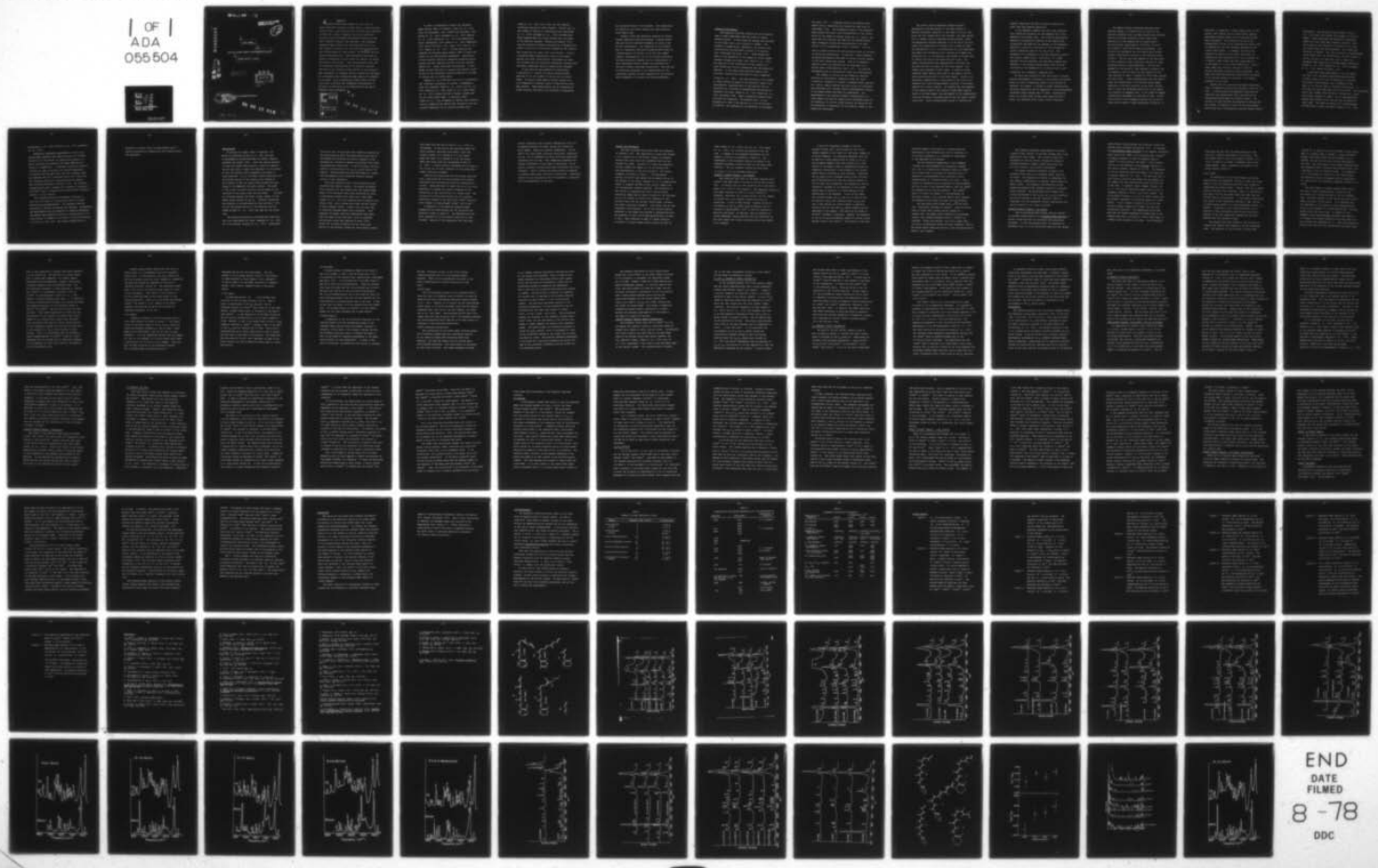

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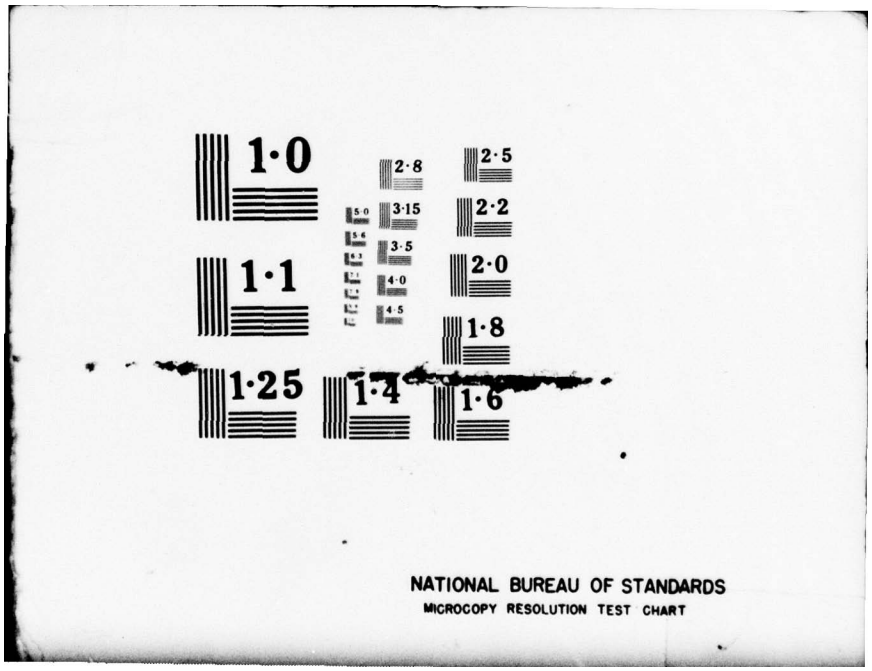
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(9) FINAL REPORT.

(15) NAVAL AIR SYSTEMS COMMAND CONTRACT N00019-77-C-0190

(6) PRIMARY EVENTS IN VISION

(13) 95 P.

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(11) 16 MAY 1978

Abstract

Resonance Raman spectroscopy has been used to study chemically modified retinal analogs involving chain substitutions, ring substitutions or Schiff base linkages. In addition, retinal fragments and fully deuterated retinals were investigated, and infrared spectra of the four isomers of retinal were obtained. Low frequency resonance Raman spectra are also reported for all of the isomers of retinal, for the protonated and unprotonated Schiff bases of trans-retinal, for beta-Ionone and for trans-3-dehydroretinal. Band assignments were made to specific vibrational motions using the results of normal coordinate calculations. These investigations have led to a detailed understanding of the spectral features observed in the resonance Raman spectra of the retinylidene chromophore in rhodopsin and our results form the basis for interpreting the informative Raman spectra of rhodopsin in live eyes. Such investigations should allow, for the first time, a detailed and fundamental understanding of the basis of variations in the sensitivity of the eye to different laser wavelengths.

In order to understand in detail the resonance Raman spectra (RRS) of rhodopsin (Lewis et. al., 1973; Lewis and Spoonhower, 1974, Oseroff and Callender, 1974; Sulkes et. al., 1976; Callender et. al., 1976; Mathies et. al., 1976; Mathies et. al., 1977) and bacteriorhodopsin (Mendelsohn, 1973; Lewis et.al., 1974; Mendelsohn, 1976; Marcus and Lewis, 1977; Lewis, 1977; Aton et. al., 1977; Campion et. al., 1977), we have obtained and compared the resonance Raman spectra (RRS) of model compounds for the retinylidene chromophore. Specifically we have studied a series of chemically modified retinal analogs and have combined this data with the infrared spectra and normal coordinate calculations. In this manner we have been able to localize the possible origin of many of the vibrational features observed in the RRS of retinal and its Schiff bases.

Robeson et. al.,(1955) were the first to investigate the vibrational properties of retinal. Subsequently Rimai and coworkers (Rimai et. al., 1971a; Gill et. al., 1971; Heyde et. al., 1971; Rimai et. al., 1971b; Rimai et. al., 1973) studied the RRS of retinal isomers and the all-trans Schiff base. In addition, Rimai (Rimai et. al., 1973) attempted to explain their observations by comparing the results they obtained to the calculated frequencies for an infinite polyene chain

(Rimai et. al., 1973; Tric, 1969) and the observed vibrational spectra of other polyenes. Further work on the isomers of retinal in crystalline form (Cookingham et. al., 1976; Callender et. al., 1976) provided more model compound data but little insight into the origin of the spectral features. Warshel and Karplus (1974) used the potential surfaces calculated by a semiempirical method to derive the vibrational frequencies and relative RRS intensities for trans- and 11-cis-retinal. Finally, a resonance Raman investigation using 11-cis-retinal analogs with butyl substitutions (Cookingham and Lewis, 1977) showed that the scattering frequencies observed from the methyl sidegroups could be unequivocally assigned.

In this work we have extended our preliminary investigation to include the entire resonance Raman spectrum of all the isomers of retinal and its Schiff bases. To analyze these spectra retinal analogs with two fundamentally different types of modifications have been studied. These modifications can be classified as either altering the ionone ring structure or perturbing

the isoprenoid chain of the molecule. The presentation of results on the retinal analogs has been organized along these lines.

Figure 1 shows the structural formulas of retinal, several chemically modified retinals, a retinal Schiff base and the two structural fragments of retinal that we have investigated. For comparison we also present RRS of a chemically modified protonated Schiff base and infrared spectra of retinal in its four most common isomeric configurations. All of the results obtained on the above molecules together with an investigation of fully deuterated retinal isomers were analyzed with the aid of normal coordinate calculations. This has led to significant progress in our understanding of the vibrational spectra of model compounds for the retinylidene chromophore of rhodopsin and bacteriorhodopsin.

Materials and Methods:

The chemically modified retinals and 11-cis-retinal used in these experiments were the gifts of several generous donors. In most cases the analogs were received in the trans- form or as a mixture of isomers. The methods of isomerization, separation, purification and identification are described in detail in an earlier work (Cookingham and Lewis, 1977). These methods are based on the separation of the isomers by liquid chromatography (Rotmans and Kropf, 1975; Ebrey et. al., 1975). The identity of the conformation of the isomers of a given analog was initially determined from the absorption spectrum. In the cases where published spectra were available, identification was made by direct comparison (Planta et. al., 1962). The identification of the isomers by other authors was based on the absorption spectra and photochemical characteristics of the molecule such as extinction as a function of isomerization and ability to recombine with opsin (Kropf et. al., 1973; Nelson et. al., 1970; and Kropf, 1976). When no absorption spectra were available for comparison, the measured ratio of the absorption at λmax to the absorption at 254 nm was used to identify the conformation (as described in Cookingham

and Lewis, 1977). A complete table of the spectral parameters used in identifying the isomers has been given by Cookingham (1978). The fingerprint region of the resonance Raman spectra was shown to be characteristic of the conformation of the isomer of retinal (Rimai et. al., 1971a). We found that this was also the case for the analogs because their fingerprint region had characteristics that were similar to the corresponding retinal conformer. This was used to further confirm the identifications made on the basis of absorption spectroscopy. In addition, nuclear magnetic resonance measurements were made on certain critical isomers of the butyl substituted analogs. Some of the measured chemical shifts are tabulated and are discussed in an earlier paper (Cookingham and Lewis, 1977). Our previous assignments were all confirmed by these nmr investigations.

The isomers of retinal and the chemically modified retinals are photolabile, especially under the laser illumination necessary to observe a resonance Raman spectrum. As a result, sample handling and data collection techniques were developed that included liquid chromatographic analysis of the sample before and after all experiments. All measurements of the relative isomeric concentration of a given sample were made by comparing the integrated areas of the absorption vs elution volume patterns and adjusting for the difference in absorption for the specific isomer at the observation wavelength.

The retinal analog compounds presented special experimental problems because of the small amounts of material available, generally on the order of 500 µg, from which the four isomers had to be isolated. For some analogs not all isomers could be isolated or even existed. After the separations had been effected, 20 to 40 µgm of the pure isomer was concentrated to dryness with a stream of argon and redissolved in 20 µl of acetonitrile for each experiment. The sample was transferred with a syringe to a sample cell consisting of a 1.5 mm capillary tube closed at one end with an optical flat and at the other end with a cork. The sample concentrations were approximately 0.005 molar for the mono-cis and 0.01 molar for the trans isomers. Two samples were prepared of each isomer. A series of three resonance Raman spectra were taken on one sample, and one short spectrum was taken of the other sample. Little or no detectable isomerization or destruction of the retinal was observed upon liquid chromatographic analysis of the sample exposed for a short interval. By studying the time sequence of any impurity band in the series of three Raman spectra taken on the same sample, the development of any isomerization could be followed in the Raman spectra and the impurity bands identified. Liquid chromatography served to identify the

isomeric impurities and their relative concentrations after they were observed spectrally.

In the desmethyl samples long term laser radiation also caused the conversion of the aldehyde form of the samples to the alcohol form. This could be monitored by measuring the absorption spectrum of the sample after illumination and observing any changes in the absorption spectrum of retinal in the region of the blue-shifted alcohol absorption peaks at 310 and 330 nm. The appearance of a broadening of the main absorption band of the retinal isomers after a long series of 3 Raman spectral runs was detected, and some small changes were observed in the resonance Raman spectra that could be attributed to the appearance of an alcohol form in the 5-desmethyl and 9-desmethyl compounds only.

We found that acetonitrile was the solvent of choice because photochemical conversion to the alcohol was approximately an order of magnitude slower in this solvent as compared, for example, to hexane or carbon tetrachloride. The above observation can be understood if this photochemical conversion occurs through the $\eta\pi^*$ state. Acetonitrile which is a highly polar solvent would stabilize such a state, and therefore would inhibit alcohol formation.

The sample handling techniques described above required changes in concentration as a part of the analysis procedure after the Raman spectra were obtained. Thus for a further verification that large scale destruction of the samples was not taking place, a dilute solution (approximately 10^{-4} molar) of one of the isomers of each analog sample was prepared. The solution was introduced into two sample cells without further concentrating relative to the remaining stock solution. The two cells were exposed to the same level of laser radiation under conditions identical to those maintained during a Raman experiment. One cell was exposed for the period of time required to rapidly scan the spectrum, and the other cell was exposed for the time required to obtain a sequence of three Raman spectra for that analog. Liquid chromatography was then used to assay the concentration of the retinal analog sample remaining in the cells relative to the stock solution. Table I shows the percentage destruction of the sample as a function of exposure time. In all cases the sample concentration decreases as a function of time. In the case of both 13-cis-5-desmethyl-retinal and 13-cis-13-butyl-retinal where a larger percentage of material is

destroyed or isomerized, a large change occurs in the shorter time interval and at longer times a smaller percentage increase occurs as if an equilibrium were being approached. 11-cis-retinal was run at two concentrations to see if any concentration effect could be observed but the rate of sample decrease did not change significantly. In summary, the worst case estimates of sample loss by either destruction or isomerization are characterized by the 18 percent and 13 percent losses during the short experiments in 13-cis-5-dmR and 13-cis-13-BuR respectively. Even in these cases only one small shoulder on the C=C retinal stretching vibration could be detected. Only bands that could be definitely identified as belonging to the retinal analogs are labelled in the spectra presented in this work.

Crystalline all-trans-N-retinylidene-n-butylamine (NRB) was prepared as previously reported (Sulkes et. al., 1976). N-retinylidene-n-butylammonium hydrochloride (N+RB HCl) was crystallized from a solution of the unprotonated Schiff base in acidified ethanol. The solution of trans N+RB HCl was prepared by bubbling HCl gas through an ethanolic solution of the unprotonated Schiff base until the absorption specturm showed complete

protonation. The protonated Schiff bases of the 13-cis, 11-cis and 9-cis isomers were formed in a similar manner. Trans N+RB CH_3Br was prepared by condensing CH_3Br with solid trans NRB, forming a solution. This was allowed to sit overnight in the dark at -15°C and the excess CH_3Br was then evaporated. The residue was dissolved in ethanol. The final solutions of the protonated Schiff bases were 0.2M concentration, and were flowed at 2ml/sec through a 2mm capillary tube to obtain the resonance Raman spectrum with minimum sample destruction (Hirsch et. al., 1976; Mathies et. al., 1976; Callender et. al., 1976; Marcus and Lewis, 1977).

A resonance Raman spectrometer of our own design was used in all the Raman experiments described here. A krypton laser at 647.1nm was employed to illuminate the retinal analog samples, and a rhodamine 6G dye laser at 637.3nm was used to irradiate the retinals. In each case the laser beam power was 45 milliwatts. The protonated Schiff base isomers were excited at 476.2nm with 7 milli-watts from a krypton ion laser. All of the crystalline samples were excited at 647.1nm with two milliwatts of laser power. The remaining details of our spectrometer are described in detail elsewhere (Sulkes et. al., 1976;

Cookingham et. al., 1976; Collins et. al., 1977; Perreault et. al., 1976).

Temperature dependence measurements on the 11-cis-retinal Raman spectrum were made by cooling a 0.07 molar solution of 11-cis-retinal in acetonitrile sealed in a quartz capillary and observing the Raman spectrum as a function of temperature. A Lakeshore Cryotronics Spectrim cryostat was used to maintain the desired temperature. All spectra were excited at 647.1nm with a krypton laser and observed with $2cm^{-1}$ resolution and a $1cm^{-1}$ step size. The integrated areas were calculated from the digital data collected according to Durant's rule. Two different standards were used to measure the increase in intensity -- the $918cm^{-1}$ band of acetonitrile and the $1576cm^{-1}$ ethylenic stretch in 11-cis retinal.

The infrared absorption measurements reported in this work were made on a Perkin-Elmer model 621 double beam grating spectophotometer. The reference beam was attenuated to achieve the maximum pen response. Consequently the measurements are not quantative in absolute absorption. The samples were prepared by placing a single drop of 0.8 molar retinal in ethanol on an Irtran II plate and allowing it to dry to a thin film. The retinal samples were re-

dissolved in ethanol after the measurements and LC analysis indicated no isomerization had occurred during the experiment.

Calculations:

To analyze the normal modes of vibration, the Wilson FG technique (Wilson et al., 1955) was used as programmed by Schachtschneider and Snyder (Schachtschneider and Snyder, 1963). Since the retinal molecule is large for normal mode analysis, a series of approximations were made to reduce the complexity of the problem so that the program could accommodate the number of atoms specified. All of the methyl groups associated with the polyene portion of the molecule were treated as point masses as were the substituted hydrogen and butyl groups in the desmethyl and butyl analogs. The atoms in the ionone ring were treated as point masses at the locations of the carbon atoms and the masses included the attached hydrogen and methyl groups (except for the methyl group attached to the C_5). Internal coordinates were defined in the same manner as Rice and Gavin (1971) did for the polyene portion of the molecule, and the scheme of Neto et. al., (1967) was used for the ionone ring.

The relative positions of the atoms were taken from the x-ray coordinates for trans- (Hamanaka et. al., 1972) and 11-cis-retinal (Gilardi et. al., 1972). Coordinates

for 9-cis- and 13-cis-retinal were formed by rotating the all-trans-molecule about the appropriate double bond by 180 degrees and relieving any steric hindrance of the hydrogens by a further 15 degree out-of-plain twist about the appropriate single bond. 11-cis-12-s-trans- was also formed by rotating about the C_{12}-C_{13} single bond by 102 degrees. These manipulations were performed on a laboratory minicomputer with a molecular graphics program written in this laboratory.

The force constants used in the calculations were collected from several sources. The single and double force constants for the carbon-carbon and carbon-oxygen bonds on the isoprenoid chain were calculated by using a relationship between bond length and π bond order (Suzuki et. al., 1973) and inserting this in Gordy's rule (Gordy, 1946), which relates bond order and bond length to force constant values. Gordy's rule was also used to calculate hydrogen-carbon force constants. Force constants for bends, wags and interactions were taken from the study of Rice and Gavin (1971) on polyenes. Out-of-plane values were supplied by Gavin (1977). Values for the force constants for the ionone ring portion of the molecule, except for the stretching modes,

were taken from the work of Neto el. al., (1967) on cyclohexene. In the case of the stretching modes the force constants calculated from Gordy's rule were slightly higher (9.17 instead of 8.7) for double bonds and lower (3.98 instead of 4.38) for single bonds than those given for cyclohexene by Neto et. al. (1967). A force constant refinement was not done on the initial set of force constants in this preliminary normal coordinate treatment.

Most of the calculations for the analogs were done with a restricted set of force constants and internal coordinates that confined the molecular vibrations to a plane. Tests were done on trans- and 11-cis-12-s-cis-geometries to compare the errors introduced by the restriction of the calculations to a single plane. The inclusion of out-of-plane motions did not introduce significant changes in any mode above 1100cm^{-1} and only minor changes in those between 1000cm^{-1} and 1100cm^{-1}.

A comparison of the observed resonance Raman frequencies for trans-retinal and the calculated frequencies is shown in Table II. The description of the major component of the calculated vibrations is also included. Because of the assumptions that were made,

certain vibrations such as methyl deformations could not be modelled because the methyl groups were treated as point masses. These are labelled "unmodelled." Notice that in all cases modes involving single bond stretching are too low in frequency and those involving double bond stretching are too high. This immediately suggests that further refinements in this type of calculation can be made by suitably altering single bond and double bond force constants. Table II should aid future workers interested in pursuing such normal coordinate calculations. Additional results of the calculations will be discussed in connection with the presentation of the data.

Results and Discussion:

The data collected during this study are presented in Figures 2 to17. The organization is such that Figures 2 to 5 contain all of the retinal analogs with perturbations that occur along the isoprenoid chain and are arranged by isomer. Figures 6 to 9 show the respective retinal isomers and compare all of the analogs that have perturbations in the ring structure. The retinal fragments are shown in Figure 11. The deuterated retinal and Schiff base spectra are arranged with all of the isomers in one figure (Figures 12, 13, 14 respectively). Figure 10 compares the RRS of each retinal isomer with its corresponding infrared spectrum. The discussion of the results is organized such that a single retinal vibrational Raman band or group of bands will be discussed for all the isomers of retinal with reference to the pertinent data from the analogs, Schiff bases, infrared and normal coordinate treatment. This format will be used throughout the discussion. All of the solution spectra presented in this paper were studied in acetonitrile with the exception of the 9-cis-13-dmR spectrum which is shown in CCl_4. When reference is made to a Raman vibration of retinal in $CCl_4$the reader should consult the work of

Rimai (Rimai et. al., 1971a; Gill et. al., 1971; Heyde et. al., 1971), all of which was verified in our own work. A summary of the suggested assignments for the isomers of retinal is presented in Table III. The subheadings in the discussion section are keyed to the suggested assignment column of Table III for easy cross-reference. The low frequency crystalline data shown in Figure 17 will be discussed separately.

<u>1620cm^{-1}--1700cm^{-1} Region: C=O stretch</u>

The vibrations that occur in the Raman spectrum above 1600cm^{-1} have been shown to be due to the end group vibration. In retinal this is a C=O vibration and in the Schiff bases it is a C=N^{+} or C=N vibration. The remarkable feature of this Raman vibration is that it is very constant in frequency (1656-1659) for a given solvent, for all isomers of retinal and in all retinal analogs with only two exceptions. In both 13-dmR analogs, (Figures 2F and 3F) the removal of the C_{13} methyl group correlates with an increase of approximately 17cm^{-1} in the C=O vibration in both CCl_4 and CH_3CN. In addition, the C=O vibration is solvent dependent (CH_3CN:1655-1659 and CCl_4:1666-1668) as one would expect for a polar end group such as the oxygen of an aldehyde.

A small but consistent increase in the C=O stretch of about $2cm^{-1}$ can be noted for all 13-cis-isomers compared to the other cis- and trans- isomers of a similar compound. No noticeable frequency shift can be seen for a butyl substitution at either C_9 or C_{13}. This evidence suggests that butyl groups play a role similar to the methyl groups in determining either the electronic distribution in the polyene or the allowed normal mode of vibration at this frequency. Replacing the C_{13} methyl with a hydrogen alters either the electronic distribution such that the electron density in the C=O bond (and consequently the frequency of the C=O vibration) increases or the frequency of the allowed normal mode of vibration is altered because of the reduced mass of the hydrogen. In the 13-cis-isomer, the cis-bend between the C_{13} and the C=O bond as well as possible single bond rotations about C_{14}-C_{15} may have a much smaller but similar effect in partially isolating the C=O bond from C_{13} methyl effects and the remainder of the polyene. This could be the cause of the $2cm^{-1}$ increase in frequency. However, the frequency of the C=O in fully deuterated isomers of retinal occurs between $1637cm^{-1}$ and $1641cm^{-1}$. This effect is less than

one would expect on the basis of a simple deuterium substitution, and indicates that the C=O vibration is not totally isolated but is connected in some manner to the remainder of the polyene.

The C=O stretching frequencies in the fragments β-ionone and crotonaldehyde are interesting (Figures 11A, 11B). In β-ionone this frequency occurs at 1668cm^{-1} and is lower than an isolated C=O vibration which is observed in crotonaldehyde where relatively little delocalization of π electron density can occur. Thus, a higher C=O vibration would be expected in crotonaldehyde and one or both of the observed bands at 1684 and 1695cm^{-1} are probably due to the C=O vibration. The 1668cm^{-1} band in β-ionone and 1695cm^{-1} in crotonaldehyde are both more intense in the infrared spectra which supports the suggestion that these two bands are C=O stretches.

The protonated Schiff bases have a $C=\overset{H}{\overset{|}{N}}{}^{+}$ end group and a vibrational frequency at 1655cm^{-1}. When the Schiff base is formed from a methyl halide instead of a halogen acid, the methyl group attached to the nitrogen causes a mass induced frequency shift to 1630cm^{-1} as expected. Unlike the retinals, the 13-cis isomer does not have a consistently higher end group frequency. The unprotonated Schiff bases are similar to the protonated Schiff bases in this respect.

The infrared vibrational measurements all exhibit a band at about 1660cm^{-1} as the most intense band in the spectrum for each isomer. This vibration should be attributed to the C=O stretch, because the nature of infrared absorption causes a highly polar bond such as a C=O to have a very strong IR absorption.

The normal coordinate treatment indicates a C=O vibration that occurs at a frequency higher than most C=C modes. The frequency calculated is independent of the isomeric configuration or chemical substitutions for methyl groups. The frequency calculated was several hundred wavenumbers high, probably because of unrefined force constants. The calculations also suggest that a small percentage of the C_{13}=C_{14} and C_{14}-C_{15} stretching may also be coupled to the C=O vibration, which is consistent with the deuterated retinal observations that the C=O stretch is not totally isolated.

1500cm^{-1}-1620cm^{-1} Region: C=C stretch

The C=C stretching mode between 1500 and 1600cm^{-1} is the most intense band in the resonance Raman spectrum of retinal unlike the weak C=O stretching mode previously discussed. It has the greatest amount of resonance enhancement since it is the vibrational mode with the largest

Franck-Condon overlap between the electronic ground and excited states (Warshel and Karplus, 1974). In contrast, the non-resonance Raman spectrum of the fragment crotonaldehyde (Figure 11B) shows a C=O stretching mode (1695cm^{-1}) of similar intensity to the C=C ethylenic mode at 1642cm^{-1} in this molecule (see above.) This intensity profile is also observed in the infrared spectra of the retinal isomers (Figures 10A to 10E). The frequency of the C=C stretching mode does appear to be sensitive to solvent (Heyde et. al., 1971). Our data show that it also varies in position and shape with the geometry of the isomers and with substitutions along the chain and in the ring. In general, trans- isomers (Figures 2,6, 12, 13, 14) have C=C stretching modes which are low in frequency compared to 9-cis- and 13-cis- isomers. The 9-cis-isomers (Figures 3,7,12,13,14) almost always have the highest frequency C=C stretching bands for a given analog. 13-cis- isomers (Figures 4,8,12,13,14) have the next highest frequency C=C bands, and they show considerable structure. The observation can be made here that the highest frequency C=C bands occur in the isomers which have a cis bend at a point where there is a methyl group attached to the chain. This highlights

once again the fact that the methyl groups are tied into the π electron distrubution in the chain. The C=C stretch mode in the 11-cis-isomers (Figures 5,9,12, 13,14) is equal to or lower in frequency than in the trans-isomers. This is especially noticeable in the sulfur analogs (Figure 9).

Trans Isomer:

An examination of the trans-isomers where chain substitutions are predominant (Figure 2) shows very little change in the C=C stretching mode when butyl groups are substituted for methyl groups. However, when the C_9 methyl group is replaced with a hydrogen, there is a splitting in this mode, with the new peaks higher and lower than the $1577cm^{-1}$ band in trans-retinal. In the trans-13-dmR analog this region of the vibrational spectrum is very similar to trans-retinal except for a more predominant shoulder at $1568cm^{-1}$. Structure in this vibrational mode is also observed in fully deuterated trans-retinal along with a lowering of the C=C vibrational frequency as would be expected with the increase in mass.

The analogs which have changes in the ring end of retinal also exhibit some changes in the C=C stretching mode. The downshift of the C=C band in trans-5-dmR

(Figure 6B) is typical of all the isomers of this analog. When no C_5 methyl group is present, a steric hindrance between this methyl group and the hydrogen at C_8 is removed, and the ring can move into the same plane as the chain. The C_5=C_6 bond is now further conjugated with the isoprenoid chain, providing more delocalization, and a decrease in the C=C stretching mode. (A concomitant 3 to 5 nm increase in λ-max of all the 5-dmR analogs was observed as would be expected from the correlation of λ max and the νC=C stretching frequency (Heyde et. al., 1971)).

The major effect of adding another double bond to the ring in trans-3-dehydroretinal (Figure 6C) was the addition of a strong shoulder on the low frequency side. The other isomers of this analog also show considerable structure in this region. It is possible that the observation of two bands in trans-3-dehydroretinal is due to the presence of a second delocalized π electron system within the ionone ring. In support of this argument x-ray crystallography has shown that there is a 59 degree out-of-plane rotation of the ring about the C_6-C_7 single bond (Hamanaka et. al., 1972) which partially isolates the ring and chain double bonds from each other.

This is also supported by evidence from model compounds for the ionone ring. The addition of a second double bond in these model compounds, for example compare cyclohexene and cyclohexadiene, creates a delocalized π-electron system in the cyclohexadiene ring. The C=C stretching frequency in these molecules is lowered from 1653cm^{-1} in cyclohexene (Cleveland, 1943) to 1575cm^{-1} in cyclohexadiene (DiLauro et. al., 1969). However, β-ionone indicates that there is, in addition, π-electron delocalization between the ring and the chain. This molecule exhibits two C=C stretching bands at 1587cm^{-1} and 1606cm^{-1}, one from the ring and one from the chain. Both these freqeuncies are lower than one would expect for an isolated ring double bond (cyclohexene) or an isolated chain double bond (ethylene). This illustrates that there is partial delocalization of the π-electrons between the ring and the chain even in the presence of a C_6-C_7 twist similar to trans-retinal. Thus it is conceivable that the shoulder in trans-3-dehydroretinal at 1564cm^{-1} is due to a cyclohexadiene-like C=C stretch with an additional lowering of the frequency by 11cm^{-1} due to partial conjugation with the isoprenoid chain.

Another retinal analog studied was one with two double bonds in a five-membered ring that included a sulfur atom. In this molecule, the C_5-C_6 double bond and the C_5 methyl group are intact compared to unmodified retinals even though the remainder of the ring is radically different (Figure 1D). The additional double bond in the ring of the sulfur analog cannot be a part of the conjugation. Thus there is little effect on the C=C stretching mode in the trans-isomer of this analog (Figure 6D). There is an extra band seen between 1610cm^{-1} and 1620cm^{-1} in all isomers of the sulfur compound, and this may be due to the isolated C=C stretching frequency in the ring.

9-cis-Isomer

Spectra of analogs of the 9-cis-Isomer which involve chain effects are shown in Figure 3. The substitution of two butyl groups in the C_9 and C_{13} positions causes considerable broadening in the C=C band. There appear to be three bands, with two new ones at lower frequency. It is not immediately obvious what the reason for this is, but perhaps it is a mass effect which shows up particularly in 9-cis and 13-cis isomers. They also show the most splitting in the deuterated analogs. The 9-cis-9dmR shows the same splitting as

discussed earlier for the trans-isomer. The ring effects in 9-cis-isomer analogs (Figure 7) are similar to those observed in trans-isomers, with a downshift in the C=C band in 9-cis-5-dmR, structure in 3-dehydro-retinal, and a narrow, symmetric band in the sulfur analog.

13-cis Isomer

As mentioned before, all 13-cis-Isomers have structure in the C=C band (Figures 4 and 8). When a butyl is substituted in the C_{13} position, the low frequency shoulder at 1571cm^{-1} which shows up in the other spectra becomes almost equal in intensity to the band at 1581-1584cm^{-1}. In the spectrum of 13-cis-5dmR (Figure 8B) the C=C band has shifted down as in the other isomers of 5-dmR. However, in the 13-cis-isomer there is still a high frequency shoulder at 1586cm^{-1}; perhaps a bend in the chain at the C_{13} position isolates one C=C bond. The 13-cis-3-dehydroretinal has at least three bands in the C=C region, one more than in the other 13-cis-analogs. The normal two band structure of 13-cis- (one a shoulder) is seen in the sulfur analog, and the two bands are easily seen in 13-cis-deut-retinal.

11-cis-Isomer

An extra piece of information added to the study of the 11-cis-isomer is that it can be forced into a 12-s-cis-configuation with certain butyl substitutions (Cookingham and Lewis, 1977). 11-cis-13-BuR and 11-cis-DBR (Figure 5) must be in the 12-s-cis-configuration. These two analogs have lower C=C bands than the 11-cis-retinal and 11-cis-9-BuR which can also be in the 12-s-trans-configuration. The 11-cis-3-dehydroretinal has a very broad C=C band with four overlapping peaks, more than we have observed for the other addition of another double bond in the ring. It may be that the 12-s-trans, 12-s-cis possibilities also affect this. 11-cis-deut-retinal is similar to 11-cis retinal except for the lower frequency due to mass effects.

Infrared Spectra.

A comparison of the C=C stretching vibrations in the infrared spectra with the corresponding bands in the resonance Raman spectra shows the dramatic decrease in intensity of this band in the infrared (Figures 10A-10E). The different selection rules in infrared and Raman as well as the effect of resonance enhancement in the Raman should account for this observation. In trans, 9-cis- and 11-cis-retinal, the bands are very similar in infrared

and RRS. Structure is seen in the 13-cis-retinal infrared spectrum just as in the resonance Raman spectrum. There is also considerable structure in the trans-3-dehydroretinal infrared spectrum as in the Raman.

Schiff Bases.

The C=C stretching regions of the protonated Schiff bases are lower in frequency than in retinal, as would be expected with their red-shifted absorption spectra (Heyde et al., 1971). However, as in retinal and the unprotonated Schiff bases, it is the 9-cis-isomer which has the highest frequency band, with 13-cis- the next highest, and trans- and 11-cis- the lowest. The protonated Schiff base 9-cis-isomer does have some structure with a low frequency shoulder. The trans-methylated Schiff base has a C=C band similar in frequency to the protonated Schiff base.

Normal Coordinate Calculations.

The calculations of the normal modes involving double bond stretching yielded some very interesting results. In all cases six double bond stretching modes were observed. One mode was clearly the C=O stretch which was discussed previously. Four other modes are localized to one of the C=C bonds. The lowest frequency C=C mode

in all isomers contains significant contributions from all the double bond stretches. Figure 15 depicts the lowest frequency C=C stretching mode for each isomer. All of the C=C bonds are stretching in phase with an alternating pattern of extension and compression. This is the only C=C stretching mode where this pattern is present. The above mode is probably the symmetric C=C stretch that is observed as the strongly enhanced vibration at about 1575cm^{-1} in all of the retinal isomers. The previously mentioned ordering of C=C stretching frequencies is also reproduced by the calculations with the order from highest to lowest: 9-cis-, 13-cis-, trans-, and 11-cis-. The calculations predict the frequency of this symmetric stretch about 150cm^{-1} high, but the consistency of the results from isomer to isomer suggests the method gives reasonable trends. The second lowest C=C stretching frequency is always the C_5=C_6 stretch, which is reasonable in light of the constraints imposed by the ionone ring of which this bond is a part. The relative resonance enhancement of this mode for a particular geometry may account for some of the previously discussed structure within the C=C stretching region.

The computer simulations of butyl substitutions showed very little effect on the normal modes attributed to C=C stretches, in agreement with experiment except for 9-cis-DBR. However, 9-dmR and 13-dmR substitutions showed considerable changes. In the four cases of the 9-cis- and trans-isomers of 9-dmR and 13-dmR, the substitution introduced a contribution in the calculations from a carbon-carbon-hydrogen bend involving the substituted hydrogen and the nearest double bond (C_9=C_{10} for 9-dmR or C_{13}=C_{14} for 13-dmR) altering the two lowest C=C normal modes. This effect is especially interesting because either a splitting or a strong shoulder was observed experimentally in this band in the spectra of these four analogs.

<u>1300cm^{-1}-1500cm^{-1} Region: Methyl Deformations</u>.

The frequency region from 1300 to 1500cm^{-1} will be considered next because a group of vibrational bands are present which appear to have a similar origin. Unfortunately, the solvent acetonitrile masks some of the weak Raman bands of the analogs within this region; however, the CCl_4 spectra of Rimai, (Rimai et. al., 1971a; Gill et. al., 1971) reproduced in this study do show the Raman bands of the retinal isomers. The infrared spectra (Figures

10A to 10E) show considerable structure in this region and the bands are moderately intense.

C_9 and C_{13} Asymmetric Methyl Deformation.

In the infrared spectra, all of the retinal isomers and the trans-3-dehydroretinal show a single moderately intense band between $1445cm^{-1}$ and $1448cm^{-1}$. A band at a similar frequency was observed by Rimai in the Raman spectrum of each of the retinal isomers. In the 11-cis-isomer an additional Raman band was observed at $1431cm^{-1}$ which was less intense than the other $1448cm^{-1}$ Raman vibration. The original suggestion of Rimai (Rimai et. al., 1971a; Gill et. al., 1971) and coworkers that this is an anti-symmetric deformation within the C_9 and C_{13} methyl groups is probably correct, and the observations can be explained as follows. Both methyl group deformations occur at the same frequency and are degenerate in 9-cis- , 13-cis- and trans-. In 11-cis-retinal the environments of the C_9 and C_{13} methyl groups are sufficiently different because of the out-of-plane rotation about the C_{12}-C_{13} single bond (Gilardi et. al., 1972) and steric interference from the hydrogen on C_{10} or C_{12} (12-s-trans or 12-s-cis respectively) that one deformation frequency may be lowered. A similar effect

has already been shown to lower the frequency of the methyl stretch for the C_{13} methyl by 20cm^{-1} in 11-cis-retinal (Cookingham and Lewis, 1977). A second band at 1431cm^{-1} would be expected in the infrared on the basis of this suggestion. In Figure 10C the 1446cm^{-1} band is not definitely split. This may be due to the low resolution of the infrared spectrum. Further support for the antisymmetric methyl deformation can be found in compounds like acetonitrile and methanethiol which have been shown to have antisymmetric methyl deformations at 1440cm^{-1} and 1444cm^{-1} respectively (Herzberg, 1945; May and Pace, 1968). In addition, the Raman scattering from these vibrations in CH_3CN and CH_3SH is generally weak while the infrared absorption is moderately intense, as is observed for retinal (Sadtler Standard Spectra, 1973; May and Pace, 1968).

C_{13} Symmetric Methyl Deformation

For each of the four retinal isomers a band or pair of bands is present in the RRS only when a methyl group is attached to the C_{13} position. These bands are located at the following frequencies: trans-1337cm^{-1}, 13-cis-1316cm^{-1} and 1352cm^{-1}, 11-cis1345cm^{-1}, 9-cis-1329cm^{-1} and 1337cm^{-1}. In all of the butyl substituted

analogs the respective band for that isomer does not appear in either the 13-BuR or DBR analogs where the C_{13} methyl has been replaced by a butyl group. In the desmethyl analogs these bands are absent when the methyl group is removed from and present when it is bonded to the C_{13} position with the exception of the 9-cis-9-dmR analog where neither a 1329cm^{-1} or 1337cm^{-1} band appears. These bands are also present in the infrared spectra for the respective isomers at: trans-1333cm^{-1}, 13-cis-1309cm^{-1} and 1351cm^{-1}, 11-cis-1338cm^{-1} and 9-cis-1334cm^{-1}.

The 1345cm^{-1} band in the 11-cis-isomer can be seen in Figure 16 to be temperature dependent. It increases in intensity by approximately 25% on cooling from 25°C to -45°C. This increase is consistent with the increase expected in the 11-cis-12-s-trans- concentration (from 40% at 25°C to 50% at -45°C) of a 12-s-cis, 12-s-trans-equilibrium. The temperature dependence of this equilibrium was discovered experimentally with NMR measurements (Rowan et al., 1974) and later verified by calculations (Birge et. al., 1976). The resonance Raman temperature measurements indicate that the 1345cm^{-1} band is probably present only in the 11-cis-12-s-trans- conformer. The observation that the 1345cm^{-1} band is missing in all cases where a butyl group occupies the C_{13} position (Figure 5B, 5D) also supports this assignment because these molecules cannot attain the 12-s-trans- conformation with a butyl group at the C_{13} position.

In compounds containing methyl groups where definite vibrational assignments have been made, a symmetric deformation of the methyl group has been assigned to the 1376cm^{-1} and 1332cm^{-1} vibrational bands of CH_3CN and CH_3SH respectively (Herzberg, 1968; May and Pace, 1968). The retinal bands observed could be explained by assigning them as a symmetric vibration of the methyl group attached to the C_{13} position. Since these bands appear to be dependent on their environment as evidenced by the solvent effects and splitting when near the cis bend, they may be effective indications of the environment of the C_{13} methyl group.

Gem-dimethyl

The infrared spectra of all the retinal isomers contain two moderately strong bands (trans-1362, 1387; 13-cis-1362, 1380; 11-cis-1358, 1375; 9-cis-1360, 1379) which are known to be characteristic of a gem-dimethyl conformation where two methyl groups are attached to the same carbon atom (Silverstein et. al., 1974). The doublet structure is due to an in-phase and out-of-phase interaction between the two methyl groups. Since the ionone ring in retinal contains a gem-dimethyl conformation on C_1, moderate infrared bands would be observed. These bands are not observed in the RRS because the vibrations are localized near the C_1 position, which is well away from the conjugated portion of the molecule

that gives rise to the resonance enhancement of the Raman bands.

C_9 Symmetric Methyl Deformation

One other weak band is consistently observed in all retinal isomers in this region in both the RRS in CCl_4 and the infrared. This vibration is a single band in all isomers except 9-cis. In 9-cis the Raman frequencies are at 1402cm^{-1} and 1374cm^{-1} and the infrared bands occur at 1401cm^{-1} and a broad but unresolved band at 1379cm^{-1}, which also contains a contribution from the gem-dimethyl doublet. The Raman and infrared frequencies for the other isomers are: trans-1388(R), 1402(IR), 13-cis-1399(R), 1400(IR), and 11-cis-1387(R), 1382(IR). The two bands that occur in the 9-cis isomer suggest that something near the C_9 position such as the cis-bend may be causing the observed splitting.

1300-1240cm^{-1} Region: C-C-H bend + C=C or C-C Stretch

An examination of Figures 5 and 9 shows that there is a single, intense band at ~1271cm^{-1} in all 11-cis-analogs of retinal which are capable of being in the 12-s-trans-conformation. When the equilibrium between 12-s-trans- and 12-s-cis- is perturbed chemically by forcing 11-cis analogs into the 12-s-cis- conformation, (as in 11-cis-DBR and 11-cis-13-BuR) this band becomes weaker in intensity and appears to be split. This is

also the only band besides the 1345cm^{-1} band in the spectrum of 11-cis-retinal which is temperature dependent (Figure 15). This is in accord with the fact that a change in temperature also affects the 12-s-cis/12-s-trans equilibrium. Warshel and Karplus (1974) suggested that bands in this region have contributions from in-plane C-C-H bends and C-C single bond stretches. Our normal coordinate calculations indicate that normal modes which mix C-C-H deformations with C=C stretching vibrations would be observed at these frequencies. The admixture of double bond stretching would probably account for the fact that these bands are observed in the resonance Raman spectrum. Changes made at the aldehyde end of 11-cis retinal do not affect this band as is evidenced by its presnece in the 11-cis Schiff bases (Figures 13D and 14C). Changes in the ring end of the molecule in 11-cis-3-dehydroretinal and 11-cis-sulfur (Figure 9) do not change the position or intensity of the band either. However, changes in the middle of the molecule effected by a change in conformation to trans, 9-cis or 13-cis causes the band to become weaker or split. The infrared spectra of the four retinal isomers (Figure 10) confirm these observations. Raman bands in this region are not very intense in the 9-cis- and 13-cis-retinals and their analogs although some distinct features can be seen in analogs of the trans-isomer (Figure 2).

There is a consistent doublet in trans retinal and butyl substituted trans-analogs, but trans-3-dehydroretinal (Figure 6C) does not show this doublet. The additional double bond in trans-3-dehydroretinal affects the C=C stretching region and thus could also alter the above normal mode which is an admixture of the in-plane hydrogen bending and C=C stretching vibrations.

The above experimental observations locate the origin of this vibrational mode in the central portion of the isoprenoid chain. This may suggest an explanation for the single intense band in 11-cis retinal and its analogs that can exist in a 12-s-trans conformation. Retinals in this conformation approximate C_{2v} symmetry from C_5 through C_{13}. This symmetry could contribute to the intensity of the single band observed in this region of 11-cis-retinal.

It is interesting to note that the work done on rhodopsin (Sulkes et. al., 1976; Callender et. al., 1976; Mathies et. al., 1976) shows a single, high intensity band in the resonance Raman spectrum of rhodopsin at 1271cm^{-1}. Two bands were also observed at 1275 and 1296cm^{-1} in the spectrum of isorhodopsin (Mathies et. al., 1976). These compare well with what we observe in 11-cis- and 9-cis-retinals, respectively, and their Schiff bases.
Modelling of squid acid metarhodopsin (Sulkes et. al., 1976)

shows the trans-doublet at 1273 and 1287cm^{-1}. Thus, this region of the retinal spectrum appears to be very useful in modelling visual pigments and possibly even bacteriorhodopsin. On the basis of the strong 1271cm^{-1} band in rhodopsins we suggest that the conformation of the 11-cis-retinyldene chromophore in opsin is 12-s-trans. It is important to point out in this regard that rhodopsins and solutions of 11-cis Schiff bases both lack a 1345cm^{-1} band. This is the only other vibrational mode in 11-cis-retinal that showed a significant intensity increase as the temperature was lowered and the concentration of the 12-s-trans conformer increased.

1240cm^{-1}-1100cm^{-1} Region: Fingerprint

The region of the resonance Raman spectrum between 1100cm^{-1} and 1240cm^{-1} has been called the fingerprint region because of the distinctive spectral features that occur in this region which are characteristic for each isomer (Rimai, et. al., 1971a). Within this region of the retinal spectrum four different general types of vibrations appear to occur depending on the geometry and structure of the molecule. In this section the discussion will center on explaining how the retinal analogs were used to isolate the origin of the vibrations occurring in this region.

C-C Stretch, CH_3 Rock

The first group of bands that appears to be affected by similar structural substitutions occurs between 1180cm^{-1} and 1240cm^{-1}. The analogs with ring substitutions (Figures 6 to 9) leave this region of the spectrum virtually unchanged in all retinal isomers except for minor intensity changes. However, the isoprenoid chain substitutions affect this region dramatically. In trans-retinal the 1198cm^{-1} band shifts to lower frequency and increases in intensity (Figures 2B and 2C) when a butyl group is substituted at the C_9 position. A butyl substitution at the C_{13} position appears to have an identical effect in the DBR case but causes a band at 1187cm^{-1} in the 13-BuR case. Desmethyl substitutions have the opposite effect. They reduce the intensity of the bands in this region and cause an increase in the frequency of the observed band to 1222cm^{-1} and 1215cm^{-1} for 13-dmR and 9-dmR respectively (Figures 2F and 2E). A vibration that consists of different mixtures of C-C single bond stretching and in-plane methyl rocking of one or both methyl groups would explain this series of changes. Such motions are known to occur at these frequencies in alkanes that contain methyl groups (Dollish et. al., 1974). The reduction in frequency on substitution of butyl groups is consistent with this suggestion. Additional

evidence can be found in work on carotenoids (Rimai et. al., 1973), where the compound Amphotericin B, which has no methyl groups, has no 1010cm^{-1} band and only a very weak 1195cm^{-1} band. In order to explain our experimental observations the resonance enhancement of this methyl rocking and C-C stretching vibration must be such that it increases with the presence of butyl groups and is decreased by replacement with a hydrogen atom.

The bands in 9-cis-retinal follow a similar pattern with few exceptions, which might be explained by the presence of the cis-bend between C_9 and C_{10}. As in the trans isomers the 1201cm^{-1} band in 9-cis-retinal appears to be downshifted to 1183cm^{-1} and increase in intensity in the DBR analog (Figure 3B). In the 9-cis-9BuR analog, the presence of the cis-bend between the C_9 butyl and C_{13} methyl may alter this mode of vibration which includes rocking motions of both the butyl and methyl groups. Two separate modes each involving a rocking and C-C stretch might then be observed, one at the higher frequency associated with a methyl group and one at the lower frequency indicative of a butyl group. Indeed two vibrational bands are observed at 1187cm^{-1} and 1201cm^{-1} in 9-cis-9BuR (Figure 3C) instead of at a single lower frequency as in trans-9-BuR (Figure 2C). In the 9-cis-13-dmR analog, the intensity is again reduced and the frequency raised to

1228cm^{-1}. In 9-cis-9-dmR the combination of the reduced intensity and the cis-bend is sufficient to make the band unobservable or to completely change the character of this vibration.

In 13-cis-retinal the substitution of a butyl group at the C_9 position is the only one that significantly alters the appearance of the spectrum in this region. In the case of this substitution the frequency apparently is reduced to 1184cm^{-1} from 1193cm^{-1} as was previously observed for butyl substitutions with no adjacent cis-bends. The cis-bend near C_{13} apparently perturbs the C_{13} methyl such that it is no longer similar to the C_9 methyl rocking vibration as occurred in the case of 9-cis-retinal. In addition, the nature of the C_{13} methyl rocking and C-C stretching vibration is such that the frequency is increased to 1222cm^{-1} and substitution of a butyl group only perturbs this slightly to 1218cm^{-1} (Figure 4D). The variations in intensity that occur in Figures 4B and 4C are consistent with previously noted increases on addition of a butyl group as previously noted.

The 11-cis-isomer of retinal again has a cis-bend between C_9 and C_{13} which appears to affect the character of this methyl rocking, C-C vibration. In Figure 5B where both methyls are substituted by butyl groups, a single intense band occurs at 1182cm^{-1}, as though both the 1219cm^{-1} and

1206cm^{-1} vibrations had shifted. When only one methyl is replaced by a butyl group, a strong band between 1185cm^{-1} and 1188cm^{-1} is observed and either a weak 1206cm^{-1} (Figure 5C) or 1217cm^{-1} (Figure 5D) band appears. The manner in which these bands shift suggests that in 11-cis retinal the C_{13} methyl rock, C-C vibration occurs at 1206cm^{-1} while the 1219cm^{-1} band is due to the C_9 methyl. In an analogous manner to the previously discussed isomers the rocking motion involving the butyl groups appears most intense.

All of the vibrations discussed above are visible in the infrared spectra. They are not especially intense and therefore probably do not involve significant changes in dipole during the vibration. Thus, the infrared data does not contradict any of the suggested assignments of coupled methyl rocking and C-C stretches made above.

The normal mode calculations suggest that modes involving methyl rocking motions, methyl stretching and single bond stretching could occur in this frequency range. In the calculations, the C_9 methyl group is the methyl group with the largest contribution. In the above discussion substitutions for the C_9 methyl group had the greatest effect on the character of the Raman spectrum between 1180cm^{-1} and 1250cm^{-1}. Thus, the calculations of the character of the normal modes of vibration are in general agreement with the conclu-

sions drawn from the spectra of the chemically modified retinals.

C-C Stretch.

A consistently intense band occurs in both the resonance Raman and infrared spectra of 9-cis-, 13-cis- and trans-retinal between 1145cm^{-1} and 1165cm^{-1}. The conspicuous absence of this band in 11-cis-retinal (Figure 5A) suggests that the planar portion of the retinal from C_7 to C_{13} is involved in the normal mode giving rise to this vibration. The planar arrangement of the isoprenoid chain is significantly distorted in 11-cis-retinal. A symmetric stretch involving several single bonds in the isoprenoid chain, particularly those between C_7 and C_{13}, seems the most likely assignment for this band. Both strong infrared and strong Raman bands would be expected for such a C-C single bond stretch. The non-planar nature of the isoprenoid chain in 11-cis-retinal, which would disrupt any in-phase stretching motion of single bonds, explains the absence of this band in the 11-cis spectra. The resonance Raman intensity varies somewhat depending on the analog and seems to be less intense in the di-butyl-retinal isomers. This band becomes more intense in trans-13-dmR and trans-9-dmR. It is most intense in the trans-9-dmR isomer where an extended chain of C-C single bonds exists without an

additional perturbation from the C_9 methyl group. Further support for our assignment can be found in a very intense band at a similar frequency (1158cm^{-1}) in carotenoids which has been assigned (Rimai et. al., 1970) to a C-C stretching vibration. Our normal mode calculations suggest that such a C-C single bond stretch could occur at approximately this frequency.

In all analogs studied, the 9-cis isomer has a significantly lower C-C stretching frequency at about 1147cm^{-1} compared to trans- and 13-cis (Figures 2,3 and 4). This observation is interesting in view of the previous observation that the C=C stretching frequency for 9-cis-retinal was the highest of all the retinal isomers. This effect would be expected for single and double bond stretches if less delocalization occurred and can be taken as additional evidence supporting this assignment.

C_{14}-C_{15} Stretch.

All of the 9-cis-, 11-cis- and 13-cis-isomers of retinal and the analogs (except 9-cis-13-dmR) have a band that occurs between 1114cm^{-1} and 1133cm^{-1}. This band is also present in the infrared spectrum with increased intensity and as in the Raman it is the strongest in 13-cis-retinal. No vibrational band is present in the Schiff bases (except for the 9-cis NRB in which the situation is complicated by a low C-C stretching frequency for retinals and Schiff bases) which suggests that the

aldehyde group in retinal is involved. Aliphatic aldehyde groups are known to have C-C stretching vibrations associated with the carbon-carbon single bond adjacent to the carbonyl group. The frequency of this stretching vibration occurs between 1120cm^{-1} and 1090cm^{-1} (Dollish et. al., 1974). Since electron delocalization causes the C=C stretch to be somewhat lower in retinal (1650cm^{-1}) than in aliphatic aldehydes (1920cm^{-1}), the C_{14}-C_{15} single bond stretch of the aldehyde group would be expected to occur at a somewhat higher frequency than in aliphatic aldehydes. In support of this assignment the cis-bend appears to be important in determining the intensity. The closer it is to the aldehyde group, the stronger the C_{14}-C_{15} stretch at about 1120cm^{-1}. In trans-retinal, no Raman vibration is observed; however, a strong infrared band is observed for trans-retinal at 1110cm^{-1}.

11-cis-retinal has one additional band that occurs at 1143cm^{-1} in all 11-cis- analogs with the exception of the sulfur analog. The infrared spectrum has a moderately intense band at 1140cm^{-1} and the 11-cis-protonated/unprotonated Schiff base does not have any vibrational band at this frequency. The absence of this vibration in the sulfur analog with the different ring structure suggests this vibration may be due to a single bond stretch in the ring that also contains single bond stretches in the isoprenoid chain near the ring. In the Schiff

bases this mode may not be present or may not be resonance enhanced.

In many instances, the resonance Raman scattering from visual pigments and bacteriorhodopsin can be better modeled with a Schiff base spectrum than by the spectrum of the corresponding retinal isomer. The suggested assignments for retinal may be useful in interpreting the origin of some of the bands in the fingerprint region of the Schiff bases. In the Schiff bases the 1140cm^{-1} to 1170cm^{-1} band may be due to a single bond stretching motion of the Schiff base isoprenoid chain between C_9 and C_{13} similar to that observed in retinal. In an analogous manner the structure between 1180cm^{-1} and 1215 cm^{-1} in the Schiff bases may be due to vibrations involving methyl rocking motions and C-C single bond stretching motions as in retinal.

The Schiff bases do exhibit one vibration that is not present in any of the retinals or model compounds. This vibration occurs at approximately 1236cm^{-1} in all isomers of the protonated Schiff bases (Figure 13) and at approximately 1224cm^{-1} in the isomers of the unprotonated Schiff bases (Figure 14). The Schiff bases differ from retinal in that they contain a butyl group attached through a protonated Schiff base to the C_{15} carbon of the polyene (Figure 1C). The 1236cm^{-1} band and the 1224cm^{-1} band are probably due to motions involving

the Schiff base nitrogen. This is supported by the fact that upon deprotonation of the Schiff base this band shifts from $1236cm^{-1}$ to $1224cm^{-1}$. This shift is similar to that observed for the C=N stretch. Further evidence to support this suggestion is found in the spectrum of a trans-methylated Schiff base (Figure 13A) where the methyl group is attached to the nitrogen. The $1237cm^{-1}$ vibration appears split into two vibrations at $1237cm^{-1}$ and $1251cm^{-1}$. The $1236cm^{-1}$ vibration is very prominent in the rhodopsin and isorhodopsin spectra of bovine visual pigments (Mathies et. al., 1977), and this assignment should be useful in interpreting the visual pigment spectra.

$990cm^{-1}$-$1030cm^{-1}$ Region: $C-CH_3$ Stretch.

There is one prominent band between 990 and $1030cm^{-1}$ in all of the retinal isomers except for 11-cis-. The band is found at $1009cm^{-1}$ in trans- $1009cm^{-1}$ in 9-cis-, and $1012cm^{-1}$ in 13-cis-retinal. In 11-cis-retinal, two bands are seen, at 997 and $1017cm^{-1}$. Rimai (Rimai et. al., 1971) suggested that the band or bands in this region are due to chain carbon-methyl stretching modes. Work from this laboratory (Cookingham and Lewis, 1977) established that the two bands in 11-cis-retinal can be assigned as follows. $997cm^{-1}$ to the C_{13} methyl group and $1017cm^{-1}$ to C_9 methyl group. This assingment was made by substituting butyl groups for methyl groups. For example, in

11-cis DBR (Figure 5B) no bands are found in this region; however, a new band appears at 1080cm^{-1}. In 11-cis-9-BuR (Figure 5C), the 997cm^{-1} band remains, the 1017cm^{-1} band is gone, and the 1076cm^{-1} band is present at about one-half the intensity as in the DBR spectrum. This enabled the assignment to be made for the C_9 methyl group. In the 11-cis-13-BuR (Figure 5D) spectrum, the 997cm^{-1} band is gone, the 1017cm^{-1} band remains, and there is a 1083cm^{-1} band. The new band at 1080cm^{-1} cannot be considered a carbon-n-butyl stretching mode because a simple butyl mass effect would decrease rather than increase this stretching frequency. Skeletal stretching modes of alkanes (Simananonti and Mizushima, 1949; Brown et. al., 1954) occur about these frequencies, although it is difficult to understand how such a vibrational mode would be resonance enhanced. These observations are confirmed in the other isomers studied. In trans-DBR (Figure 2B) the band at 1009cm^{-1} is gone and a new band appears at 1080cm^{-1}. The 9-BuR and 13-BuR show about half intensity at 1009cm^{-1} and half at 1084cm^{-1}. The very same pattern is seen in butyl substituted 9-cis- and 13-cis-retinals (Figures 3 and 4) except the intensity of the 1080cm^{-1} band is weak when it is next to a cis-bend. The removal of methyl groups in the desmethyl retinals shows a decrease in the intensity of the 1009cm^{-1} band and no new bands appearing. In the 5-dmR spectra (Figure 6B,

7B and 8B) there is no apparent decrease in intensity of the $1009cm^{-1}$ band, indicating no contribution from the C_5 methyl. Stronger evidence for this is that when the C_9 and C_{13} methyl groups have butyl groups substituted for them there is no residual intensity at $1009cm^{-1}$ which could be attributed to the C_5 methyl (Figures 2B, 3B, 4B, and 5B).

In two separate discussions above our data suggested that the C_{13} methyl group is directly involved in a specific normal mode. The C_9 and C_{13} methyl group assignments made in this section for 11-cis-retinal further support these suggestions. As we have demonstrated above the frequency of the C_{13}-CH_3 stretching vibration in 11-cis-retinal is $20cm^{-1}$ lower than the corresponding C_9-CH_3 stretching vibration. It is encouraging to note that in 11-cis-retinal, this same effect is detected for all the vibrational modes having a C_{13}-CH_3 component in their description. These normal modes, (for example the asymmetric methyl stretch and the methyl rocking C=C stretch) were detected and previously assigned using data on all the retinal isomers. However, in 11-cis-retinal, because of the unique characteristic of the C_{13}-CH_3 group, the asymmetric methyl stretch involving the C_{13} methyl occurs at $1431cm^{-1}$ whereas a similar vibrational mode consisting of the C_9 methyl is detected at $1448cm^{-1}$. In complete analogy the C_{13} methyl-rocking, C-C stretch occurs at $1206cm^{-1}$ while the C_9 methyl

rocking, C-C stretch is observed at 1219cm^{-1}.

The Schiff bases (Figure 13) show a carbon-methyl stretching region similar to that seen in retinals. There are single bands in trans, 9-cis and 13-cis, and two bands in 11-cis-N^{+}RB. The 3-dehydroretinals also show this general scheme, but have some slight intensity at 1030cm^{-1} in the 13-cis and 9-cis isomers. There is also extra intensity between 1020 and 1030cm^{-1} in some of the sulfur analog spectra. It is not understood what this is due to, but it may be attributable to changes in the ring in these compounds or ring-methyl stretches.

The infrared spectra show very low intensity multiple bands in the carbon-methyl stretching region. All of the carbon-methyl stretching modes should show up in the infrared including ring methyls which may account for the additional infrared bands. Our normal mode calculations indeed showed a separate band with carbon-methyl stretching, and showed that this band shifted in frequency when a butyl mass was substituted for a methyl mass.

<u>950cm^{-1}-980cm^{-1} Region: C-C-H Bend, Out-of-plane</u>.

There are low intensity bands between 950 and 980cm^{-1} in all the Raman spectra of retinals and Schiff bases. The frequency and intensity of these bands appear to be unaltered by changes on the chain or ring. Absorption in this region is

very intense in the infrared spectrum, and Rimai (1971a) attributed it to out-of-plane hydrogen bends on the chain. Warshel and Karplus (1971) suggested that this band could also have contributions from the C_5 methyl stretching mode. An examination of the trans- 5-dmR spectrum (Figure 6B) shows no noticeable changes in intensity in this band when the C_5 methyl is absent; however, with such a small band it would be hard to notice intensity differences. In all 3-dehydroretinals there appears to be a high frequency shoulder at 976cm^{-1} which could be due to the C_5-methyl stretch, brought into resonance when the double bond conjugation is extended further into the ring in this analog.

950cm^{-1} to 790cm^{-1} Region

In the solution spectra of the retinal isomers and the analogs (Figures 2 to 9) a few resonance Raman bands appear at frequencies between 750cm^{-1} and 950cm^{-1}. The infrared spectra (Figure 10) also show some structure in this region. The normal coordinate calculations suggest that these vibrations may come from C=C-C bending vibrations of the polyene and will have frequencies that fall in this range. These vibrations may be the ones we observed.

790cm^{-1} and Below

Considerable theoretical work has predicted the existence of low frequency vibrations in retinyldene-like molecules (Warshel and Karplus, 1974; Warshel and Dauber, 1977). The existence of

these modes has been verified by our experiments in all of the isomers of retinal and the protonated and unprotonated Schiff bases as well as in the fragment β - Ionone. Figure 17 depicts the spectra of all of these compounds from 10cm^{-1} to 1100cm^{-1}. All of the samples are the crystalline form of these molecules with the exception of β - Ionone which is a liquid at room temperature. The spectra of the retinal isomers were also studied in solution, but the Rayleigh scattering masked the low frequency modes. Vibrations in the higher frequency region (150 to 750cm^{-1}) were weak in all cases except 11-cis-retinal.

The region below 100 cm^{-1} for each retinal isomer (Figure 17B to 17E) is quite unique. The relative intensities of the bands, the frequencies of the bands and the number of bands composing the spectrum of each isomer are different. There do seem to be some common features, such as at least one very strong band between 22cm^{-1} and 30cm^{-1} in each isomer and a second band between 34cm^{-1} and 44cm^{-1}. One other band occurs about 90cm^{-1} in all of the isomers. The remainder of the spectrum to 700cm^{-1} is very weak with a few bands occurring at the same frequency in each isomer. 11-cis-retinal has a much stronger spectrum and will be discussed in detail later. The normal mode calculations predict a large number of vibrations occurring below 700cm^{-1}. Because of the lack of infrared spectra and analog Raman spectra, very few definite assignments

can be made. In general, the calculations predict C=C-C bending modes from about 400cm^{-1} to 700cm^{-1}, torsional modes from about 400cm^{-1} to 150cm^{-1} and torsional modes coupled with skeletal modes below about 150cm^{-1}. These calculations generally agree with previous calculations (Warshel and Karplus, 1974; Warshel and Dauber, 1977).

As previously mentioned, 11-cis-retinal has additional intense bands in the low frequency region. We see relatively strong vibrations at 26, 34, 40, 58, 82, 134, 193, 260, 271, 405, 522 and 564 cm^{-1}. The vibrations at 193, 260, 271 and 564cm^{-1} are also present in solution spectra (cyclohexane solvent) and are observed at 181, 248, 259, and 560cm^{-1}. Bands of this intensity were not observed in any of the other retinal isomers. In an explanation of the source of the featureless absorption spectrum of 11-cis- retinal, Warshel and Karplus (1974) used torsional modes with ground state frequencies of 286, 202, 83, 55, 29 and 17cm^{-1} to explain how low frequency progressions wipe out all the structure in the absorption spectrum. The principle 11-cis-retinal bands that we observe are in excellent agreement with their predictions.

The resonance Raman spectrum of trans-retinal shares several common features with those of the protonated and unprotonated Schiff bases of retinal and trans-3-dehydro-

retinal. The spectra of trans-retinal and trans- 3-dehydro-retinal are almost identical with the exception of a 53cm^{-1} band, a stronger 91cm^{-1} band and 167cm^{-1} band in trans-3-dehyrdoretinal. The Schiff bases both have a strong 30cm^{-1} band but no strong bands between 40cm^{-1} and 60cm^{-1}. In addition to a 64cm^{-1} band they have a doublet structure near 100cm^{-1}, in contrast to the retinals. The protonated Schiff base also has a band at 184cm^{-1}. Few additional definite assignments can be made, but there is the suggestion that the differences between the trans-retinal spectrum and trans-3-dehydroretinal spectrum may be due to the enhancement of several ring torsional modes. The differences between retinal and the Schiff bases are probably due to the presence of the Schiff base and butyl group instead of the aldehyde group.

The β - Ionone fragment has some very interesting structure although extremely low frequency modes are obscured by Rayleigh scattering. The bands at 585, 404, 341 and 114cm^{-1} agree remarkably well with the predictions of Warshel and Karplus (1974) of 567, 391, 285 and 102cm^{-1}. The strong 88cm^{-1} band predicted was not observed because it may have been masked by the Rayleigh line.

Conclusions:

The results of this study have revealed considerable information on the particular nature of the normal modes of vibration of retinal and related models for visual pigments and bacteriorhodopsin. By studying the combined information available from the resonance Raman and infrared spectra of all the isomers of retinal, chemically modified retinals, and normal coordinate calculations, reasonable assignments for all of the vibrational features observed in the resonance Raman spectra were made. Table III contains a brief summary of the suggested assignments of the bands observed in the resonance Raman spectra of the isomers of retinal. In a few instances our results were applied to interpret the spectral features of the Schiff bases of retinal. Many of these same features were also reflected in the resonance Raman spectra of visual pigments. Thus, the results of this study should enable future workers studying visual pigments and bacteriorhodopsin to understand in greater detail the information present in the resonance Raman spectra of visual pigments.

This data should be of considerable interest to those workers who are attempting to calculate resonance Raman

spectra of retinylidene chromophores (Warshel and Karplus, 1974; Warshel and Dauber, 1977). Many of their predictions of expected low frequency modes were verified by our experimental data (Figure 17.) Further theoretical calculations on some of the butyl or desmethyl analogs may prove useful in providing additional information for refining these calculations.

Acknowledgements

The chemically modified retinals used in this study were the generous gifts of several donors. We wish to thank Prof. Allen Kropf of Amherst College for the butyl-retinal and desmethyl-retinal samples and for his suggestions on the use of liquid chromatography. 11-cis-retinal crystals were supplied by Mr. Paul Brown of Harvard University. Drs. B. Pawson, W. Scott, V. Gloor and F. Weber of F. Hoffmann-LaRoche and Co. supplied 11-cis-retinal, 3-dehydroretinal and the sulfur analog. Dr. Henri Crespi prepared and gave us totally deuterated Halobacterium halobium from which we extracted fully deuterated retinal.

This work was supported by a grant from the National Institutes of Health EY 01377 and starter grants from the Research Corporation and the American Chemical Society Petroleum Research Fund. A. Lewis is an Alfred P. Sloan Fellow. A. Lemley is an NIH postdoctoral fellow.

We also wish to thank Thomas Keffer and Gregory J. Perreault for their efforts in writing the molecular graphics program. Michael Marcus assisted in performing the infrared measurements on the retinal isomers. We also thank Dr. Bishun Khare for the use of his infrared spectrometer and for his help in making the measurements.

Table 1

Summary of Laser Destruction Tests

Sample	Exposure Time (hours)	% Destruction
11-Cis-retinal	2	1.2±0.8
(10^{-4}M)	8	8.7±1.0
11-Cis-retinal	2	1.8±1.4
(10^{-3}M)	8	10.5±2.7
9-Cis-3-dehydroretinal	1.5	4.0±2.9
	6	17. ±2.4
13-Cis-5-desmethyl-retinal	2.5	18. ±2.7
	10	39. ±2.2
13-Cis-13-butyl-retinal	2	13. ±1.1
	10	16. ±1.8
13-Cis-9-butyl-retinal	2	0. ±1.5
	10	6.2±1.4
All-trans-9-13-di-butyl-retinal	2.5	0. ±2.8
	10	4.3±2.7

Table 2

Observed and Calculated Frequencies for All-Trans-Retinal

Observed	Calculated	Description of Vibration
1656	2117	C = 0 Stretch
	2165	
	2038	
1577	1830	C = C Stretch
1568	1751	
	1719	
1448		
1388	Unmodelled	
1337		
	1533	
1282	1457	C = C Stretch
1272	1437	+C-C-H bend
	1386	
1198	1112	Some C-C Stretch
	1083	+CH_3 rocking
1163	781	C-C Stretch
Not observed	1251	ring C-C Stretch
	1112	
Not observed in trans retinal but in all cis isomers	728	C_{14}-C_{15} Stretch of Aldehyde group
1009	869	C_{13}-CH_3, C_9-CH_3 Stretch
	1017	Out-of-plane
970	954	C-C-H bends
	943	

Table 3

Suggested Vibrational Assignments

Description of Vibration	Frequencies (cm^{-1}) Trans-	13-cis-	11-cis-	9-cis-
C=O Stretch	1656	1659	1658	1656
C=C Stretch	1577 1568	1584 1573	1576	1586
C_9 and C_{13} Asymmetric Methyl Deformation	1448(CCl_4)	1448(CCl_4)	1448(CCl_4) 1431(CCl_4)	1446(CCl_4)
C_9 Symmetric Methyl Deformation	1338(CCl_4) 1387 (IR)	1399(CCl_4) 1380(IR)	1387(CCl_4) 1375(IR)	1402(CCl_4) 1374(CCl_4)
C_1 Gem-Dimethyl	1362(IR)	1362(IR)	1358(IR)	1360(IR)
C_{13} Symmetric Methyl Deformation	1337	1352 1316	1345	1337 1329
C-C-H bend+C=C Stretch or C-C Stretch	1282 1272	1282 1274	1271	1295 1280
C-C Stretch,CH_3 rock	1198	1222 1193	1219 1206	1216 1201 1187
C-C (C_9 to C_{13}) Stretch	1163	1163		1147
C_{14} - C_{15}		1118	1143 1128	1117
C_9-CH_3 Stretch C_{13}-CH_3Stretch	1009	1012	1017 997	1009
C-C-H Bend, out-of-plane (C_5 -CH_3 Strectch?)	970	969	970	963

Figure Legends:

Figure 1: (A) All-trans-retinal ($R=CH_3$). In analog compounds R=n-butyl or hydrogen. (B) 11-cis-3-dehydroretinal, (C) 13-cis-N-retinylidene-n-butylammonium hydrochloride (N+RB HCl), (D) 9-cis-(E,E,E,E)-3,7-dimethyl-9- (2.4.5-trimethyl-3-thienyl) -2,4,6,8-non-atetraenal (11-cis-sulfur), (E) Trans-crotonaldehyde, (F) β-ionone.

Figure 2: Resonance Raman spectra of (A) trans-retinal, (B) trans-9,13-di-n-butyl-retinal (trans-DBR), (C) trans-9-n butyl, 13-methyl-retinal (trans-9-BuR), (D) trans-13-n-butyl, 9-methyl-retinal (trans-13-BuR), (E) trans-9-desmethyl-retinal (trans-9-dmR) and (F) trans-13-desmethyl-retinal (trans-13-dmR) in CH_3CN. The spectral resolution is $2cm^{-1}$; all positions are accurate to $\pm 2cm^{-1}$. The solvent bands of CH_3CN have not been removed from the spectra; these bands occur at $918cm^{-1}$, $1039cm^{-1}$, $1375cm^{-1}$, $1412cm^{-1}$

and 1447cm^{-1} and are unlabeled. The apparent intensities of these bands relative to the intensities of the retinal bands vary from spectrum to spectrum, depending on the concentration of the retinal.

Figure 3: Resonance Raman spectra of (A) 9-cis-retinal, (B) 9-cis-DBR, (C) 9-cis-9-BuR, (D) 9-cis-9-dmR and (E) 9-cis-13-dmR in CH_3CN. Spectrum (E) was measured in CCL_4. A CCL_4 band is visible at 1534cm^{-1} and is not labeled. Values in parentheses for intense peaks are those measured in CH_3CN. The spectral resolution is 2cm^{-1}, and band positions are accurate to ±2cm^{-1}.

Figure 4: Resonance Raman spectra of (A) 13-cis-retinal, (B) 13-cis-DBR, (C) 13-cis-9-BuR and (D) 13-cis-13-BuR in CH_3CN. The spectral resolution is 2cm^{-1}, and band positions are accurate to ±2cm^{-1}.

Figure 5: Resonance Raman spectra of (A) 11-cis-retinal, (B) 11-cis-DBR, (C) 11-cis-9-

BuR and (D) 11-cis-13-BuR in CH_3CN. The spectral resolution is $2cm^{-1}$ and positions are accurate to $\pm 2cm^{-1}$. The weak band at $1039cm_{-1}$ is a CH_3CN solvent band visible because of the low concentration of the retinal analog.

Figure 6: Resonance Raman spectra of (A) trans-retinal, (B) trans-5-desmethyl-retinal (trans-5-dmR), (C) trans-3-dehydroretinal and (D) trans-sulfur retinal (trans-sulfur) in CH_3CN. The spectral resolution is $2cm^{-1}$, and positions are accurate to $\pm 2cm^{-1}$.

Figure 7: Resonance Raman spectra of (A) 9-cis-retinal, (B) 9-cis-5-dmR, (C) 9-cis-3-dehydroretinal and (D) 9-cis-sulfur in CH_3CN. The spectral resolution is $2cm^{-1}$, and band positions are accurate to $\pm 2cm^{-1}$.

Figure 8: Resonance Raman spectra of (A) 13-cis-retinal, (B) 13-cis-5-dmR, (C) 13-cis-3-dehydroretinal and (D) 13-cis-sulfur in CH_3CN. The spectral resolution is $2cm^{-1}$, and band positions are accurate to $\pm 2cm^{-1}$.

Figure 9: Resonance Raman spectra (A) 11-cis-retinal, (B) 11-cis-3-dehydroretinal and (C) 11-cis-sulfur in CH_3CN. The spectral resolution is $2cm^{-1}$, and band positions are accurate to $\pm 2cm^{-1}$.

Figure 10: Resonance Raman and infrared spectra of (A) trans-retinal (B) 13-cis-retinal, (C) 11-cis-retinal, (D) 9-cis-retinal and (E) trans-3-dehydroretinal. The values of the infrared band positions are accurate to $\pm 5cm^{-1}$.

Figure 11: Resonance Raman spectra of (A) trans-crotonaldehyde and (B) β-ionone. The spectral resolution is $2cm^{-1}$, and band positions are accurate to $\pm 2cm^{-1}$.

Figure 12: Resonance Raman spectra of (A) trans-deuterated retinal, (B) 13-cis-deuterated retinal, (C) 11-cis-deuterated retinal and (D) 9-cis-deuterated retinal in CH_3CN. The spectral resolution is $2cm^{-1}$, and band positions are accurate to $\pm 2cm^{-1}$. The existence of bands between 900 and $950cm^{-1}$ were verified by taking spectra in methanol which has no bands in this region.

Figure 13: Resonance Raman spectra of (A) trans-N+RB CH_3Br, (B) trans-N+RB HCl, (C) 13-cis-N+RB HCl, (D) 11-cis-N+RB HCl and (E) 9-cis-N+RB HCl in ethanol. The spectral resolution is $4cm^{-1}$, and band positions are accurate to $\pm 2cm^{-1}$.

Figure 14: Resonance Raman spectra of (A) trans-NRB, (B) 13-cis-NRB, (C) 11-cis-NRB and (D) 9-cis-NRB in CH_3CN. Samples were cooled to $15^{\circ}C$ and excitation was with ~50mw of 647.1nm radiation. The spectral resolution is $2cm^{-1}$, and band to positions are accurate to $\pm 2cm^{-1}$.

Figure 15: C=C in phase stretching vibration of (A) trans-retinal, (B) 13-cis-retinal, (C) 9-cis-retinal, (D) 11-cis-12-s-cis-retinal, and (E) 11-cis-12-s-trans-retinal as computed by normal mode calculations. The arrows represent the position of maximum displacement of the corresponding atom. The arrows are drawn twice their actual length. Any methyl group and hydrogen motions have been suppressed for clarity.

Figure 16: The temperature dependence of the vibrational bands at 1345cm^{-1} (lower) and 1271cm^{-1} (higher) in 11-cis-retinal.

Figure 17: Resonance Raman spectra of (A) trans-3-dehydroretinal (B) trans-retinal, (C) 13-cis-retinal (D) 11-cis-retinal, (E) 9-cis-retinal, (F) trans-N+RB HCl, (G) trans-N-retinylidene-n-butylamine (trans-NRB) and (H) β-ionone. All samples are crystalline except for β-ionone which is a liquid at room temperature. The spectral resolution is 2cm^{-1}, and band positions are accurate to ±2cm^{-1}.

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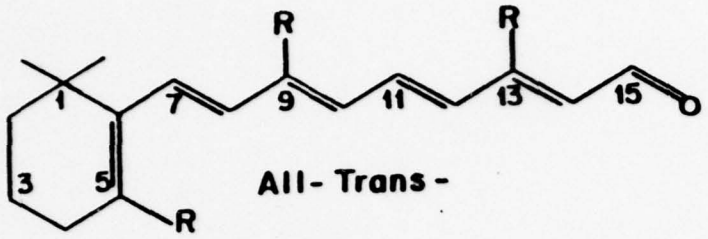

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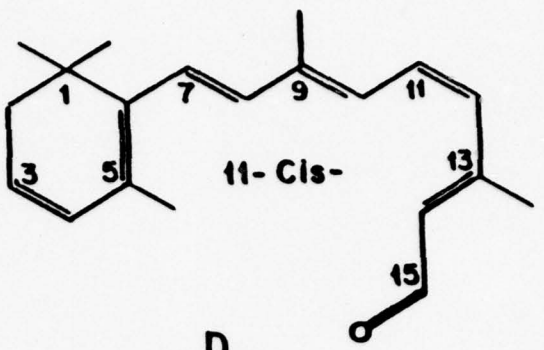

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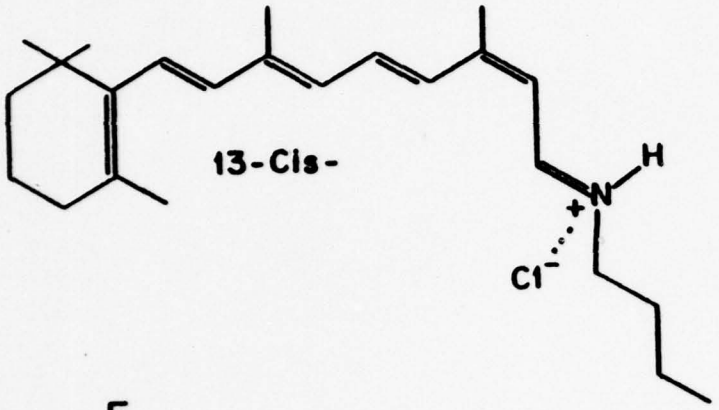

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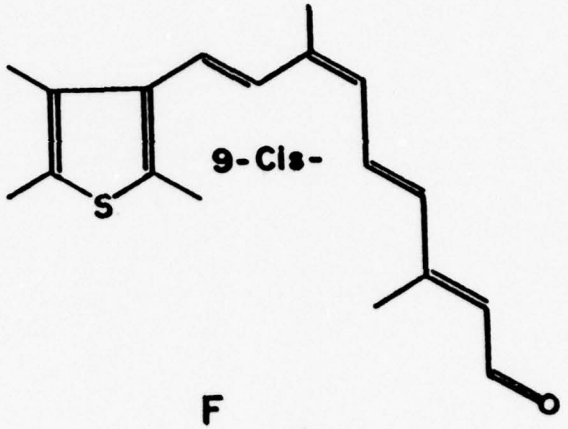

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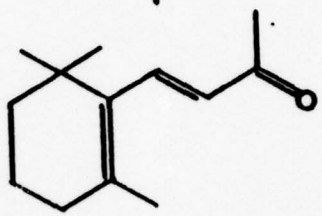

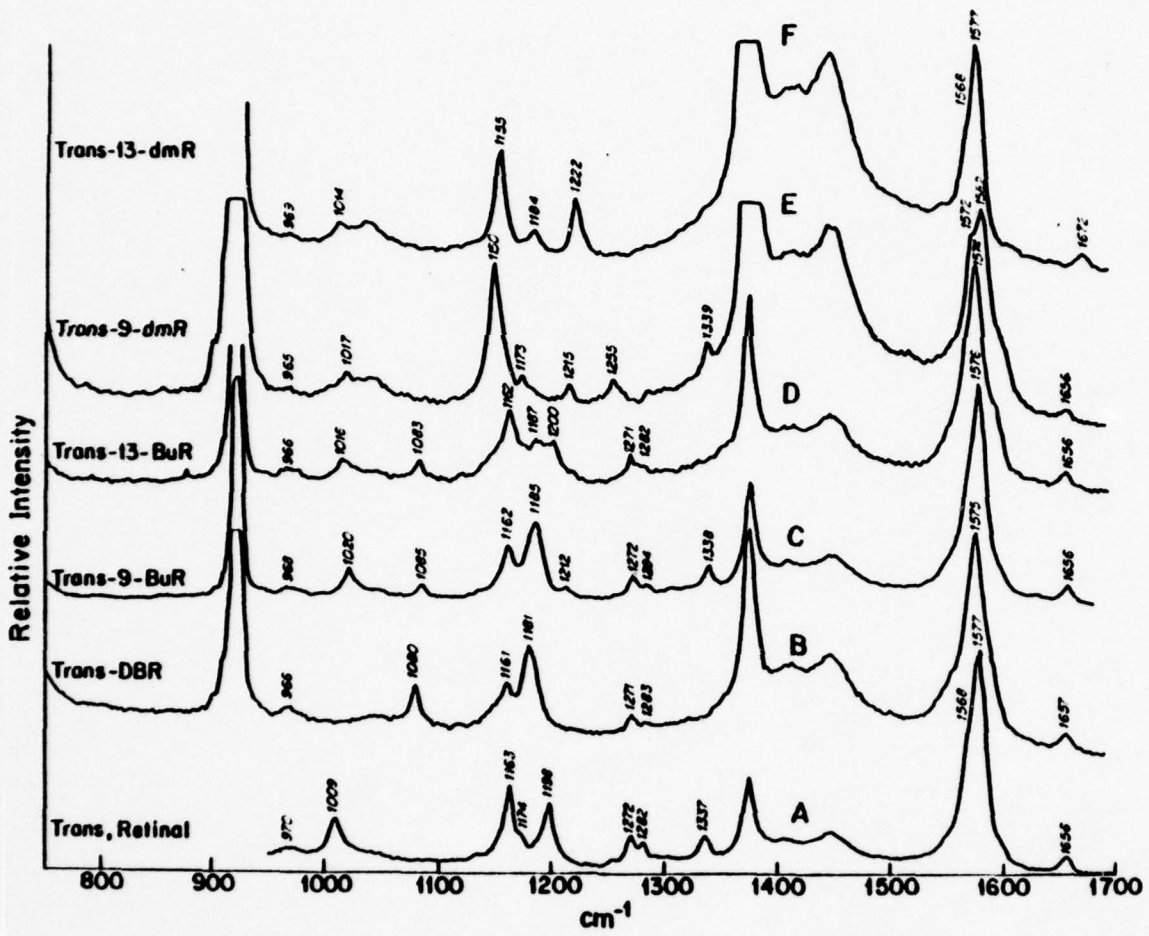
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Trans-9-dmR
Trans-13-BuR
Trans-9-BuR
Trans-DBR
Trans, Retinal
F
E
D
C
B
A
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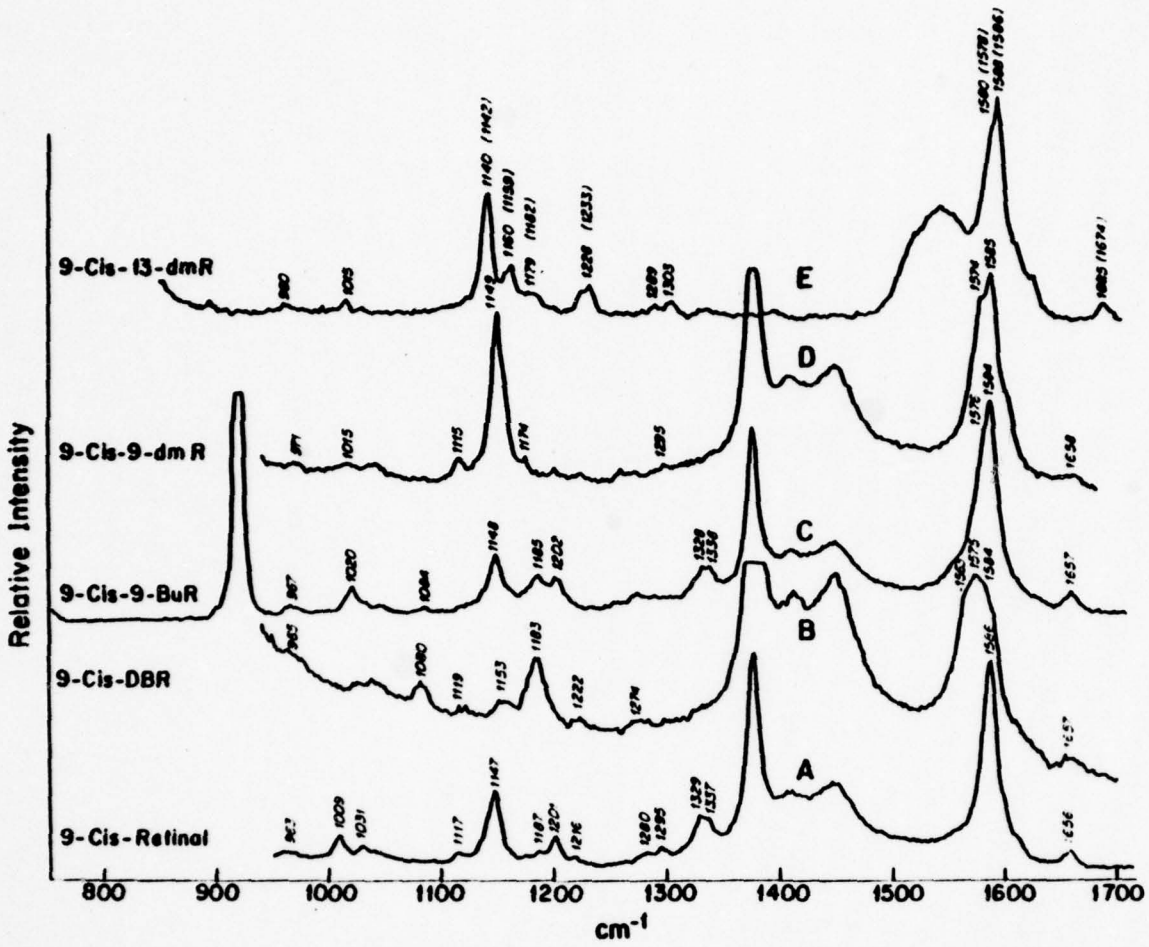
900
1000
1100
1200
1300
1400
1500
1600
1700
cm-1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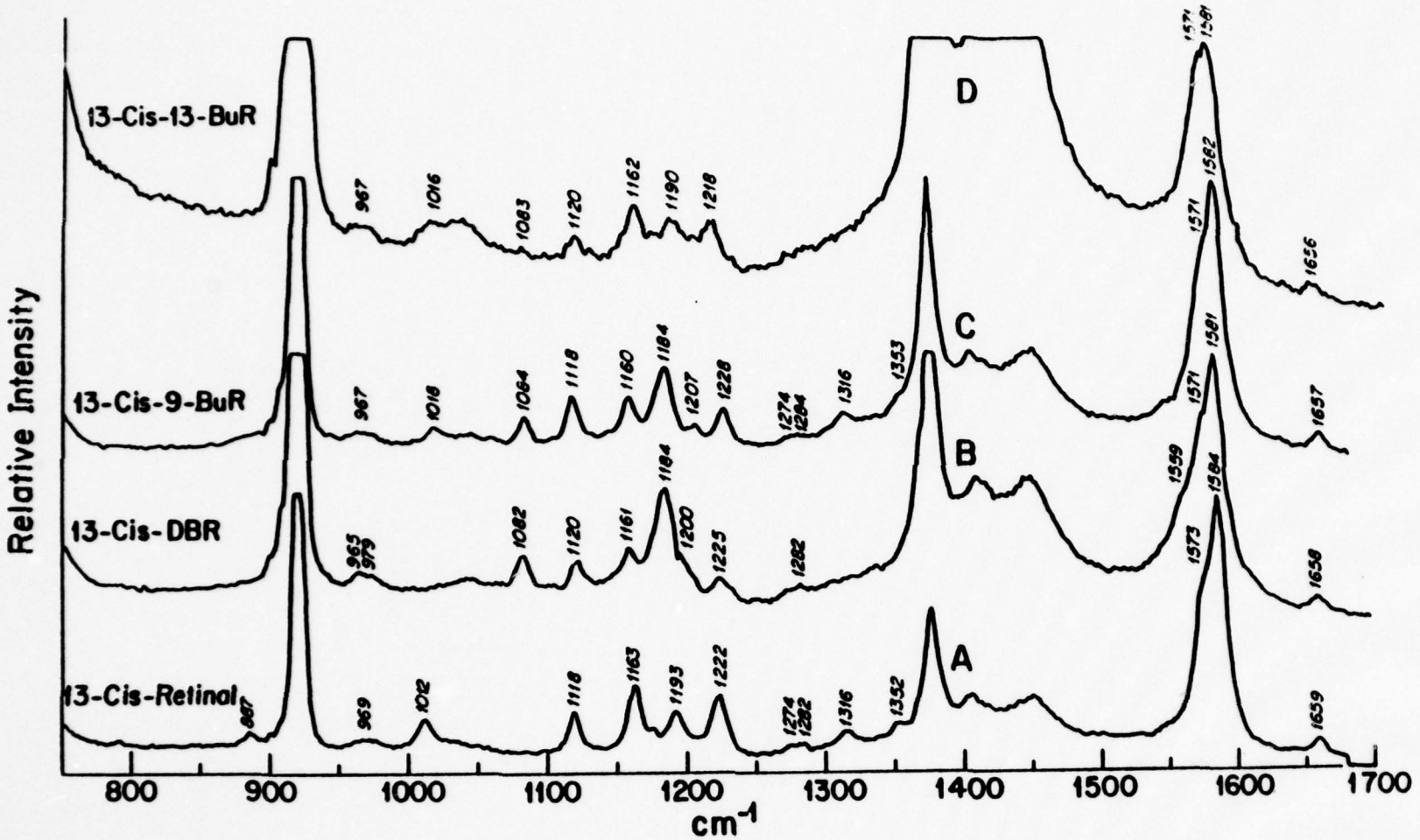
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13-Cis-9-BuR
13-Cis-DBR
13-Cis-Retinal
D
C
B
A
Relative Intensity
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1083
1120
1162
1190
1218
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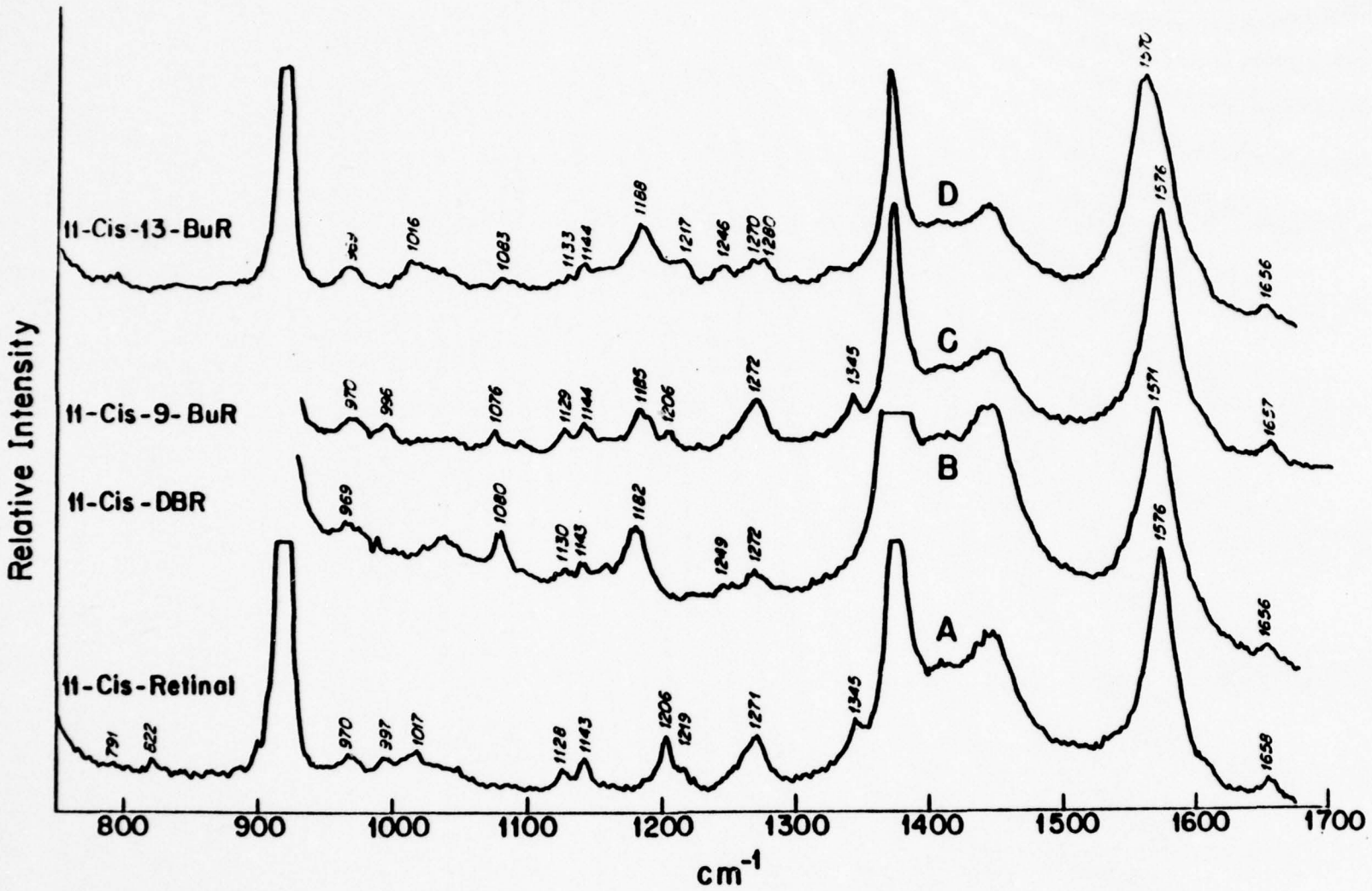
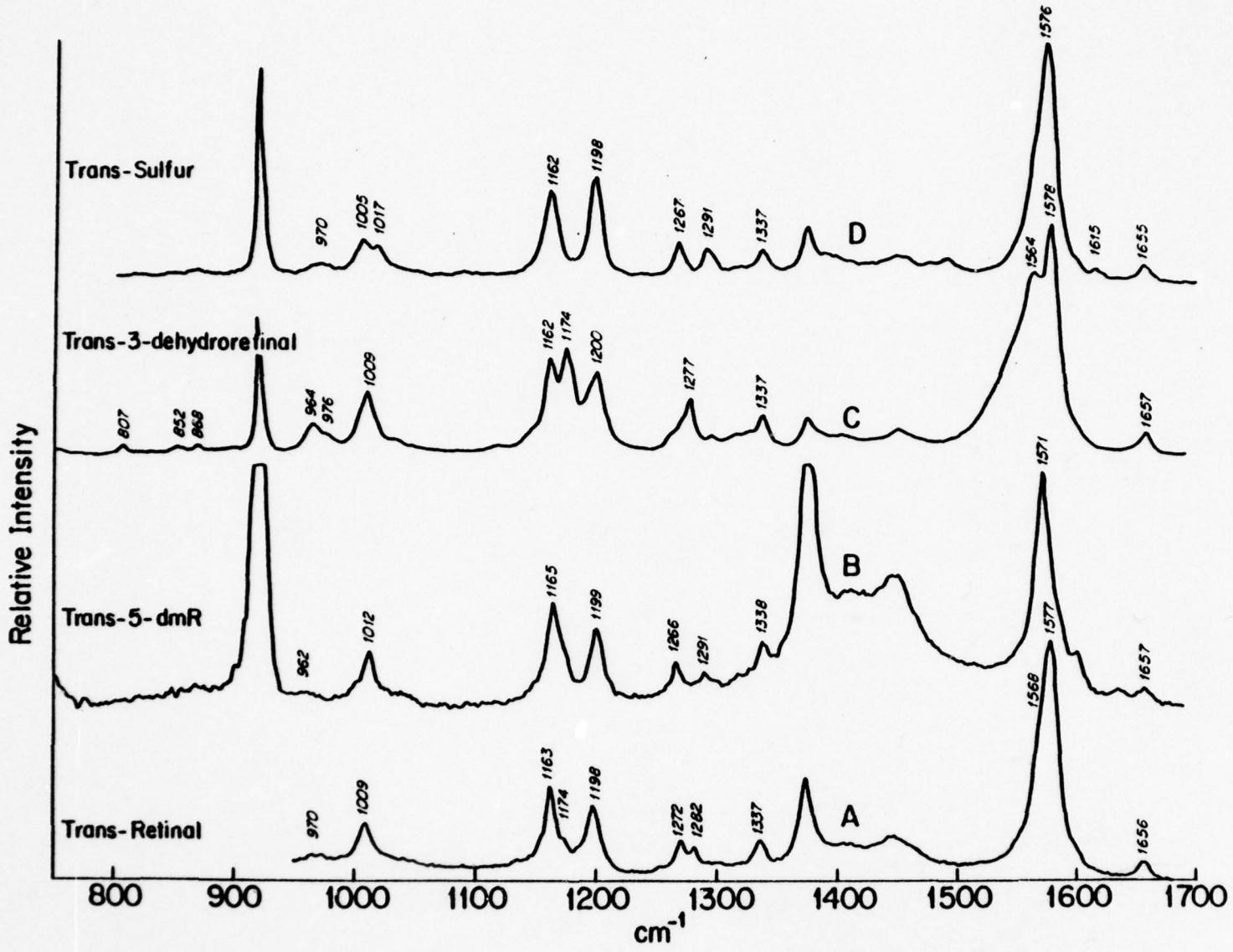
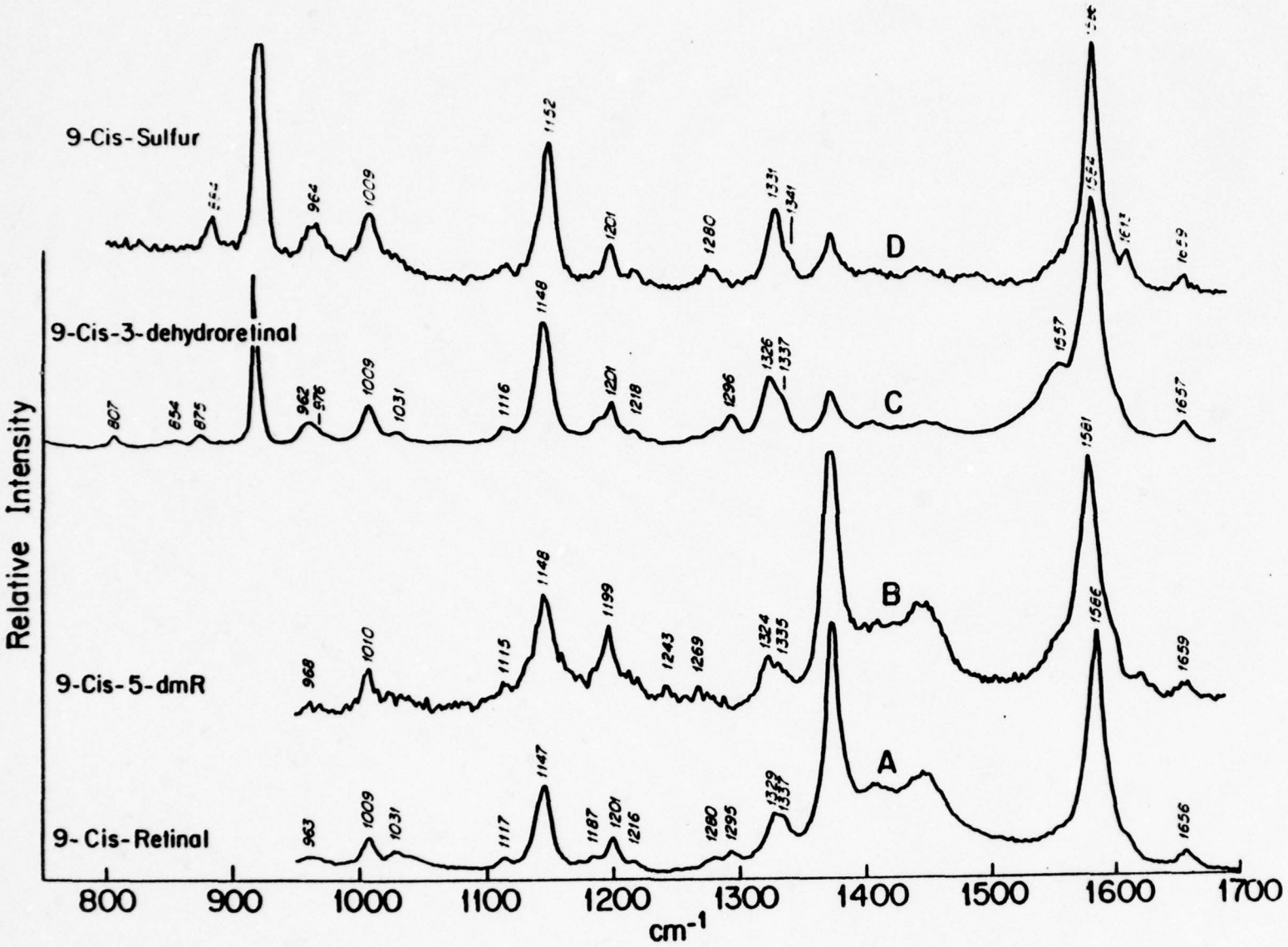
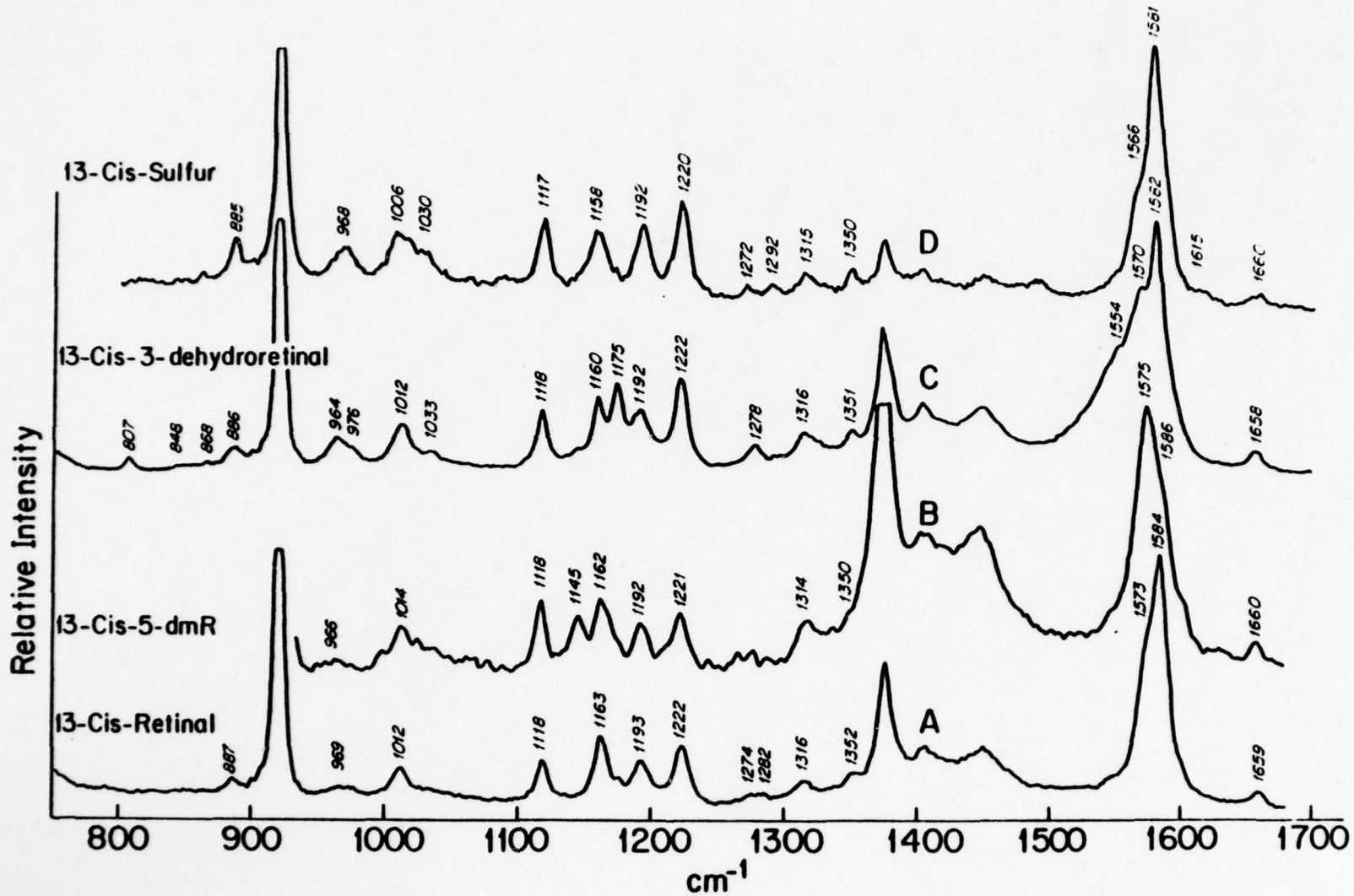
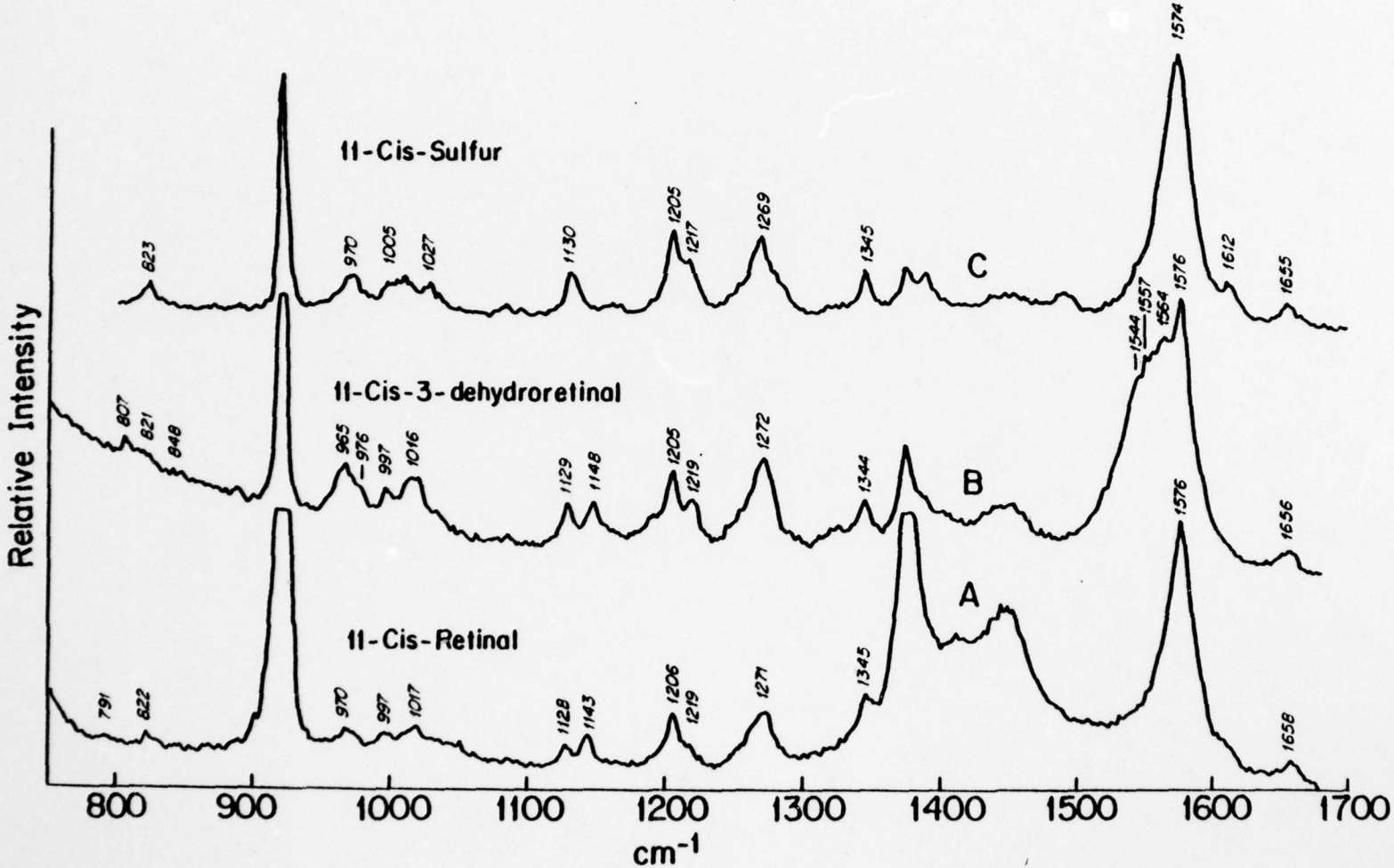
1700
cm-1

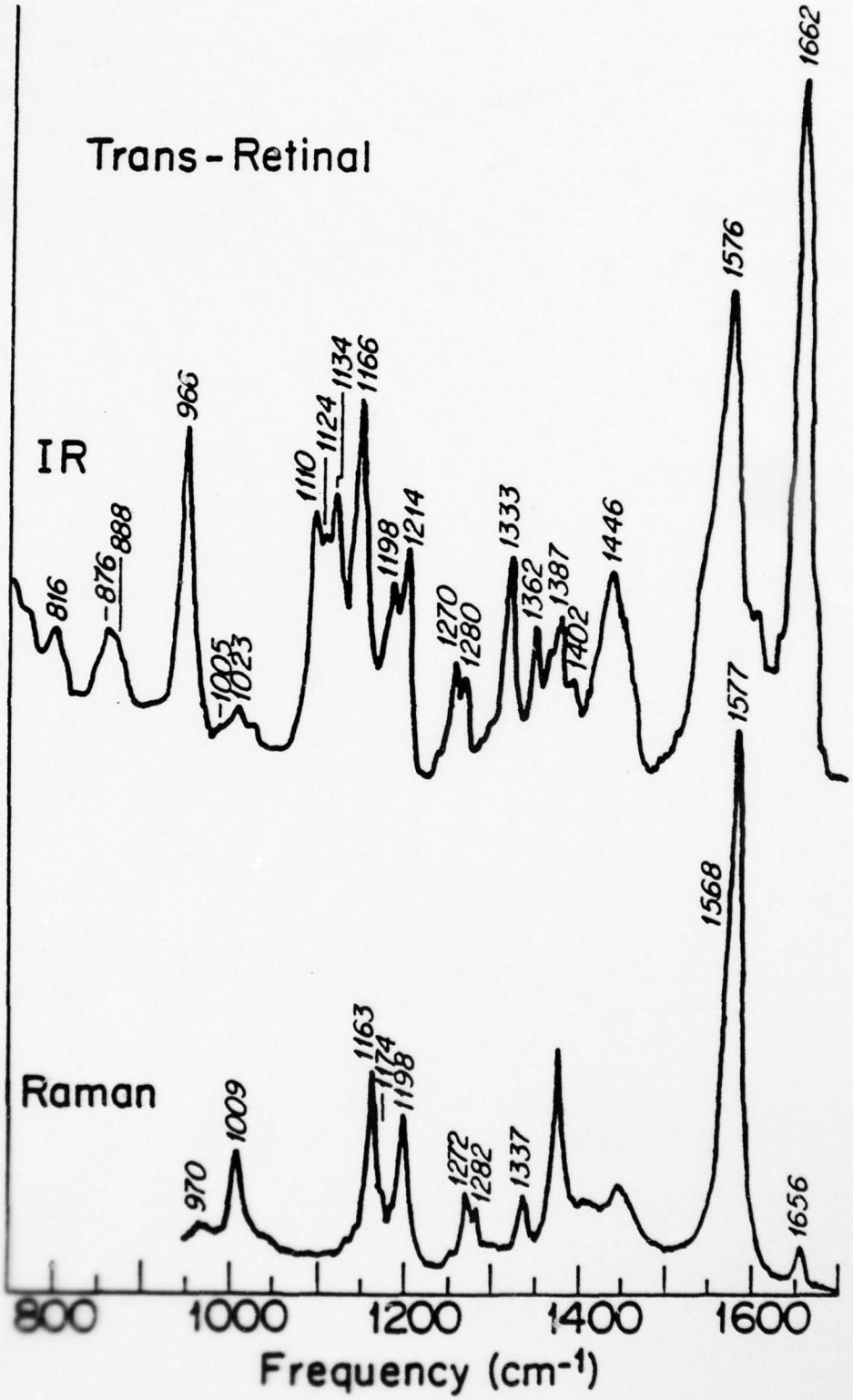

Relative Intensity
11-Cis-13-BuR
11-Cis-9-BuR
11-Cis-DBR
11-Cis-Retinol
D
C
B
A
1570
1576
1656
1016
1083
1133
1144
1188
1217
1246
1270
1280
970
996
1076
1129
1144
1185
1206
1272
1345
1571
1657
959
1080
1130
1143
1182
1249
1272
1576
1656
791
822
970
997
1017
1128
1143
1206
1219
1271
1345
1576
1658
800
900
1000
1100
1200
1300
1400
1500
1600
1700
cm-1

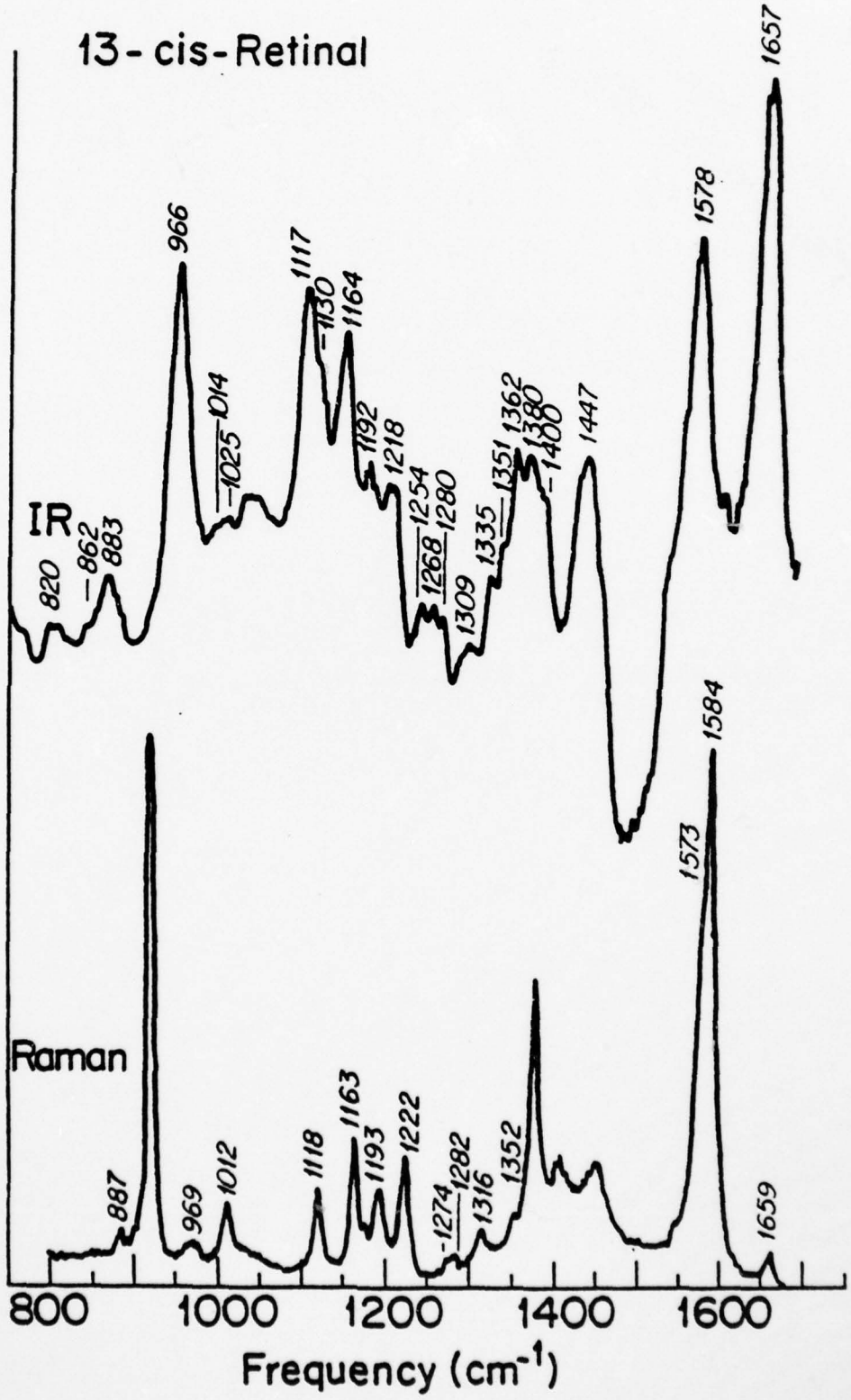

Trans-Sulfur
970
1005
1017
1162
1198
1267
1291
1337
D
1564
1576
1578
1615
1655
Trans-3-dehydroretinal
807
852
868
964
976
1009
1162
1174
1200
1277
1337
C
1657
Trans-5-dmR
962
1012
1165
1199
1266
1291
1338
B
1571
1577
1657
Trans-Retinal
970
1009
1163
1174
1198
1272
1282
1337
A
1568
1656
Relative Intensity
800
900
1000
1100
1200
1300
1400
1500
1600
1700
cm-1

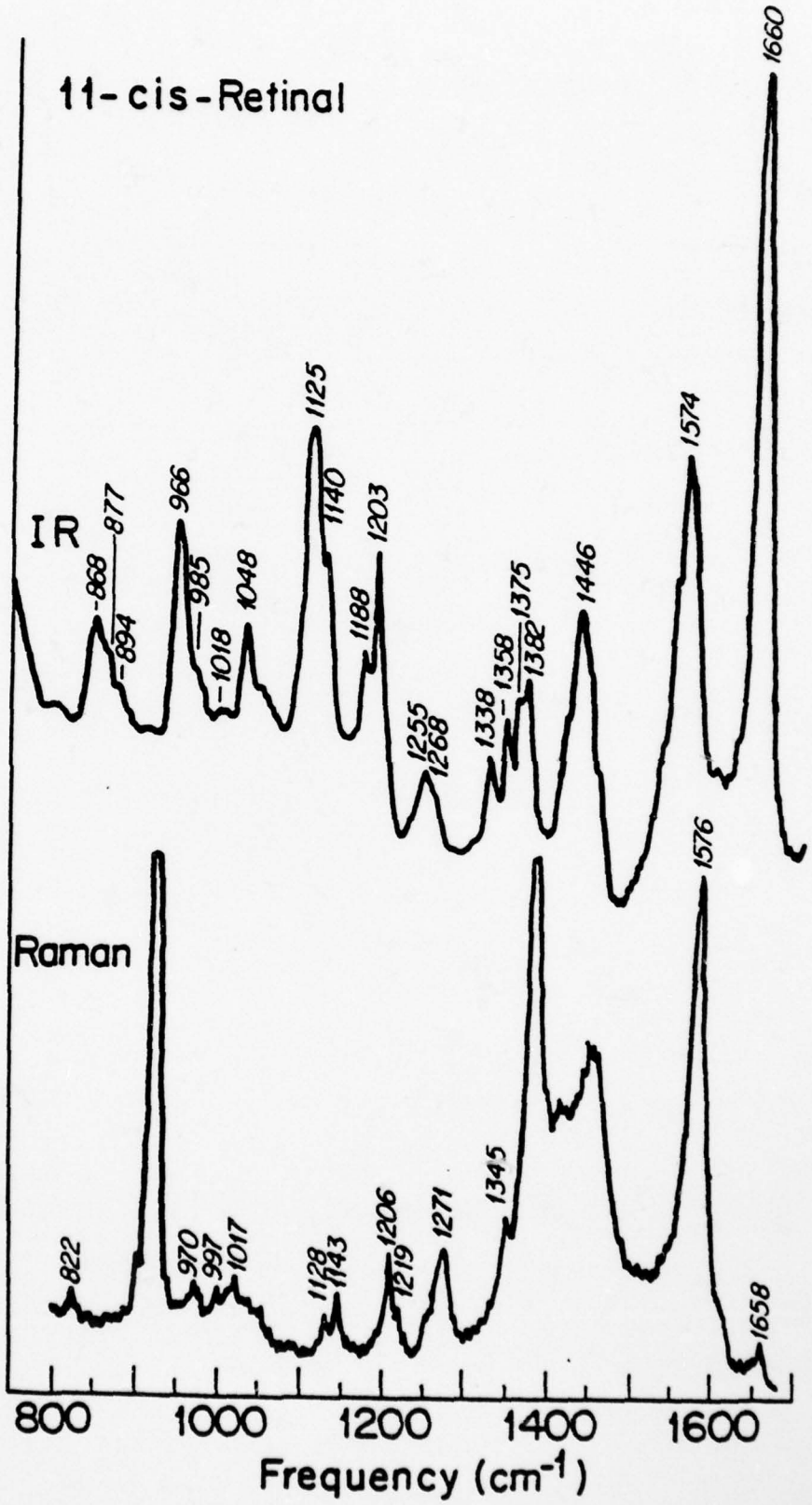

9-Cis-Sulfur
9-Cis-3-dehydroretinal
9-Cis-5-dmR
9-Cis-Retinal
Relative Intensity
cm-1
800
900
1000
1100
1200
1300
1400
1500
1600
1700
D
C
B
A
1152
1009
964
1201
1280
1331
1341
1554
1659
1148
1009
962
976
1031
1116
1201
1218
1296
1326
1337
1557
1657
807
654
875
1148
1199
1010
968
1115
1243
1269
1324
1335
1561
1566
1659
1147
1009
1031
963
1117
1187
1201
1216
1280
1295
1329
1337
1656

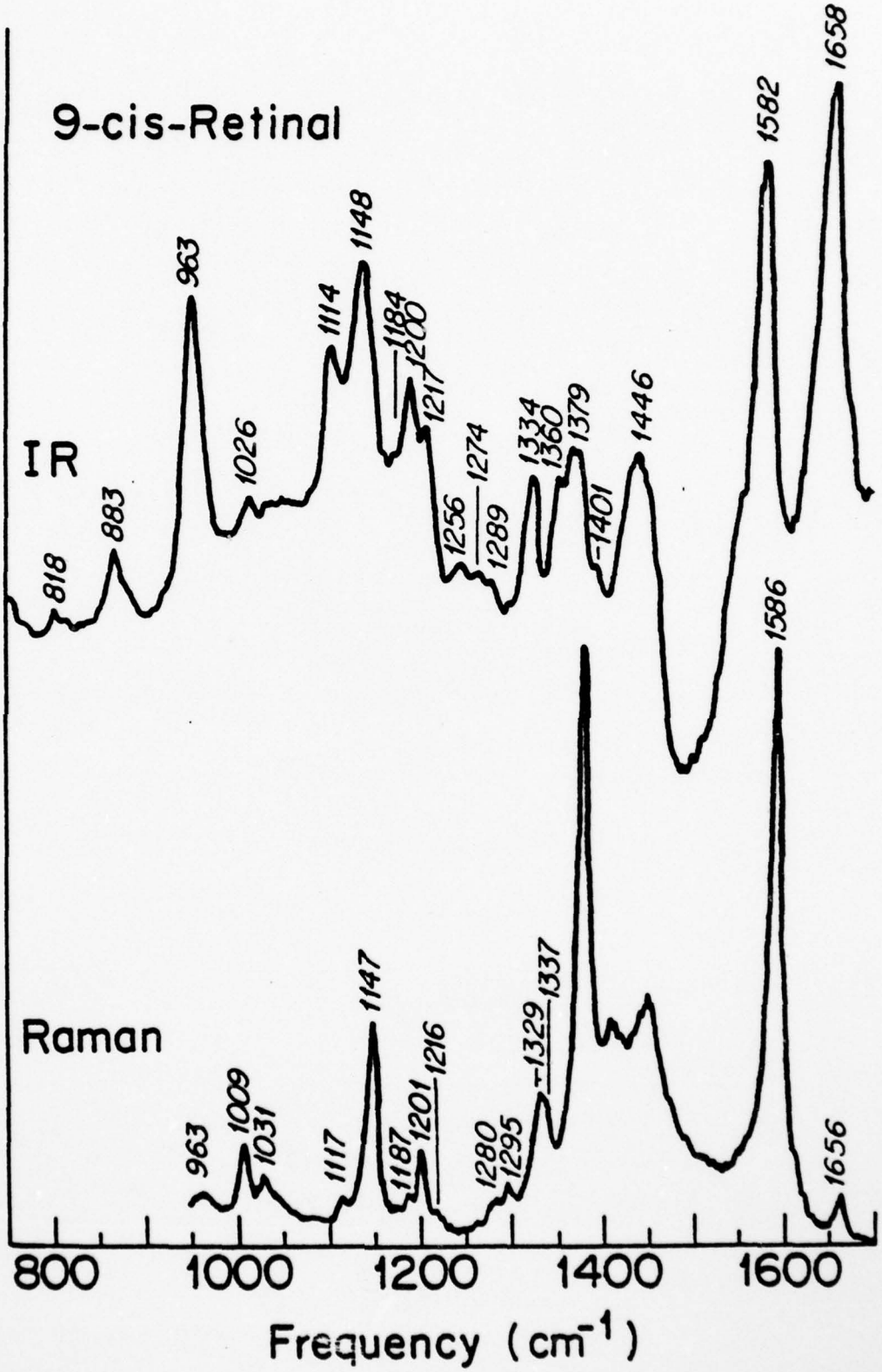

13-Cis-Sulfur
885
968
1006
1030
1117
1158
1192
1220
1272
1292
1315
1350
1566
1581
1570
1562
1615
1660
D
13-Cis-3-dehydroretinal
807
846
868
886
964
976
1012
1033
1118
1160
1175
1192
1222
1278
1316
1351
1554
1575
1586
1658
C
13-Cis-5-dmR
966
1014
1118
1145
1162
1192
1221
1314
1350
1573
1584
1660
B
13-Cis-Retinal
887
969
1012
1118
1163
1193
1222
1274
1282
1316
1352
1659
A
Relative Intensity
800
900
1000
1100
1200
1300
1400
1500
1600
1700
cm-1

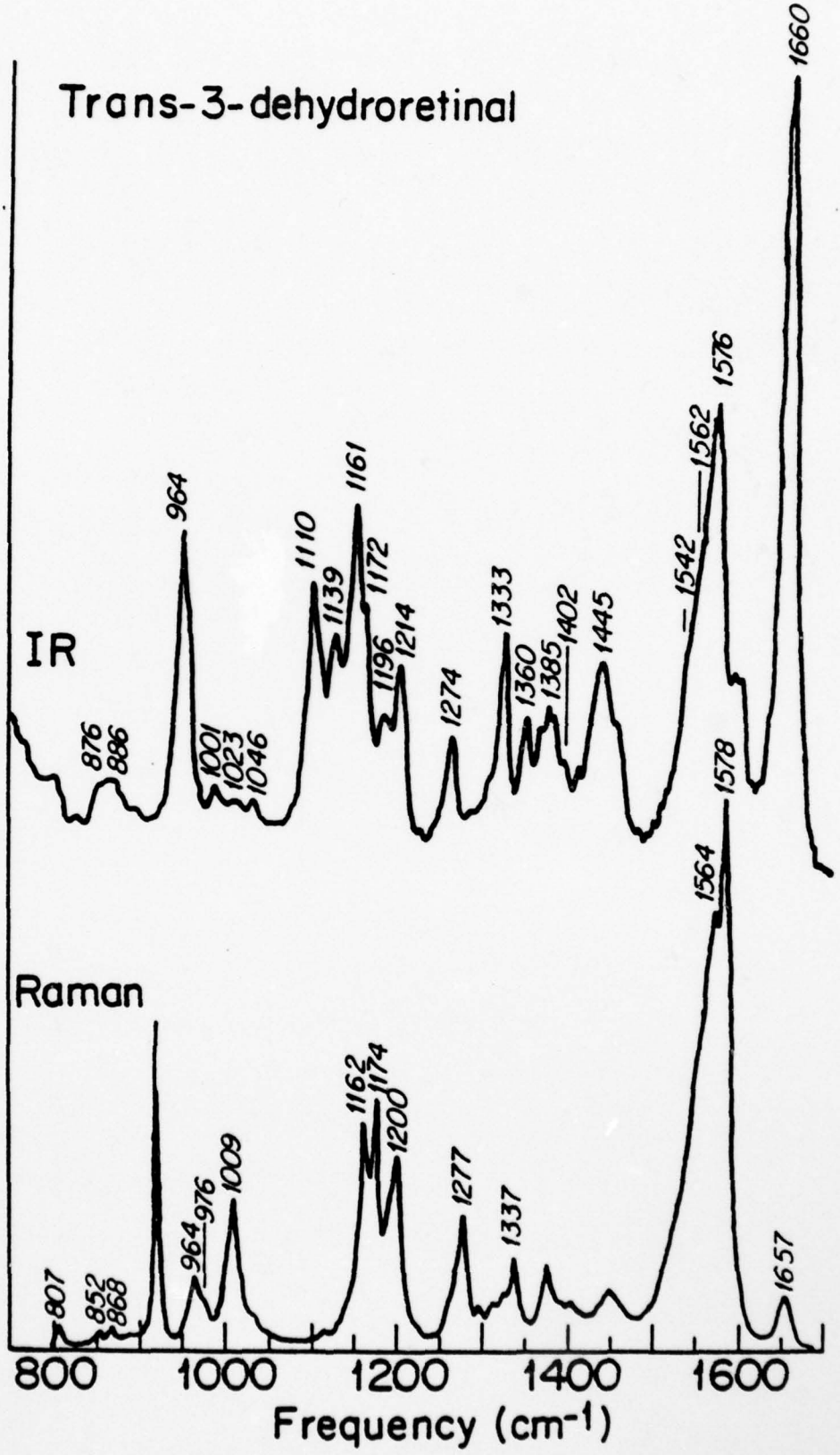

11-Cis-Sulfur
11-Cis-3-dehydroretinal
11-Cis-Retinal
Relative Intensity
cm⁻¹
800
900
1000
1100
1200
1300
1400
1500
1600
1700
A
B
C
623
970
1005
1027
1130
1205
1217
1269
1345
1574
1612
1655
807
821
848
965
976
997
1016
1129
1148
1205
1219
1272
1344
1544
1557
1564
1576
1656
791
822
970
997
1017
1128
1143
1206
1219
1271
1345
1576
1658

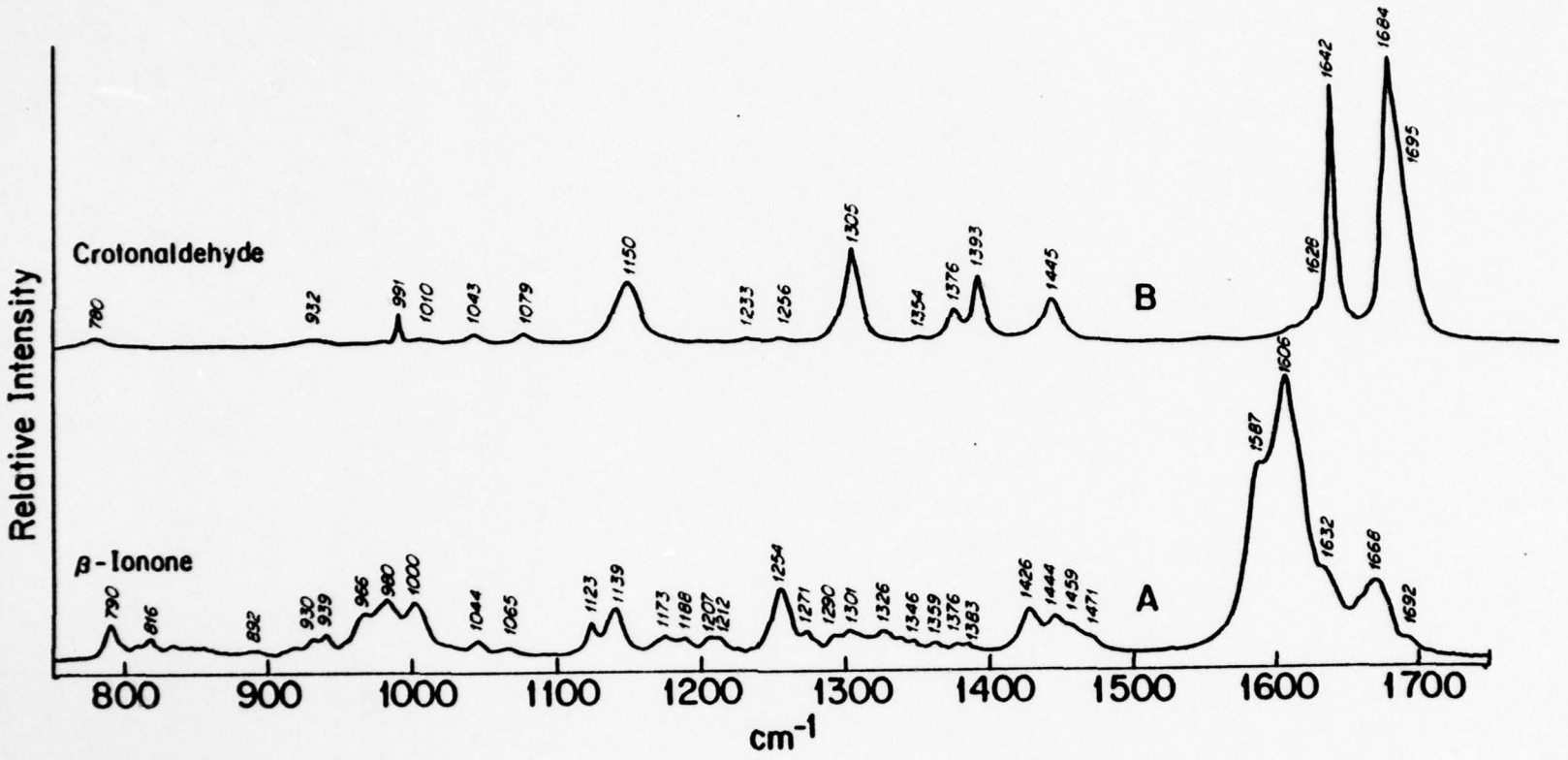

Trans-Retinal
IR
816
876
888
966
1005
1023
1110
1124
1134
1166
1198
1214
1270
1280
1333
1362
1387
1402
1446
1576
1662
Raman
970
1009
1163
1174
1198
1272
1282
1337
1568
1577
1656
800
1000
1200
1400
1600
Frequency (cm-1)

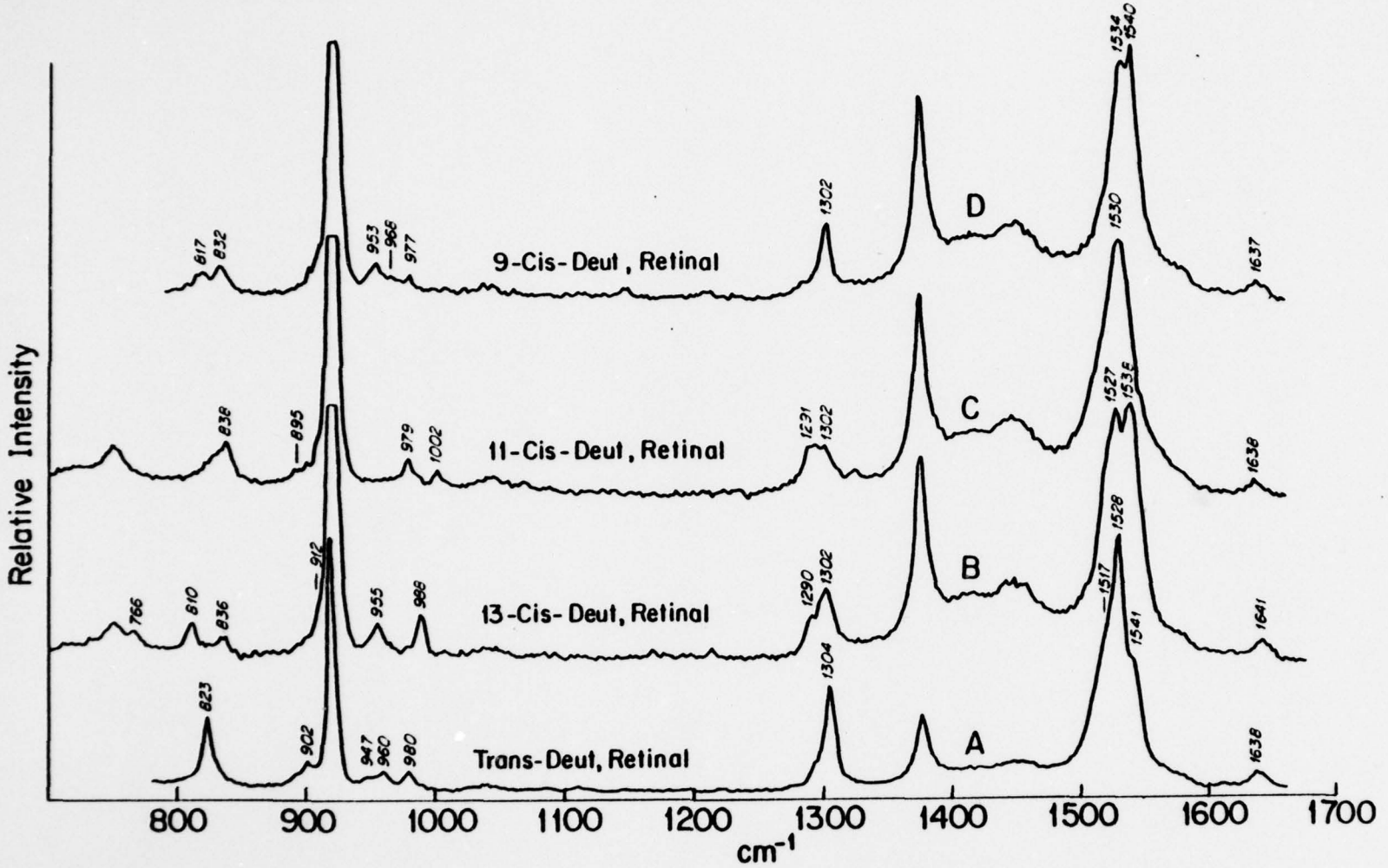

13-cis-Retinal
IR
820
862
883
966
1014
1025
1117
1130
1164
1192
1218
1254
1268
1280
1309
1335
1351
1362
1380
1400
1447
1578
1657
Raman
887
969
1012
1118
1163
1193
1222
1274
1282
1316
1352
1573
1584
1659
800
1000
1200
1400
1600
Frequency (cm⁻¹)

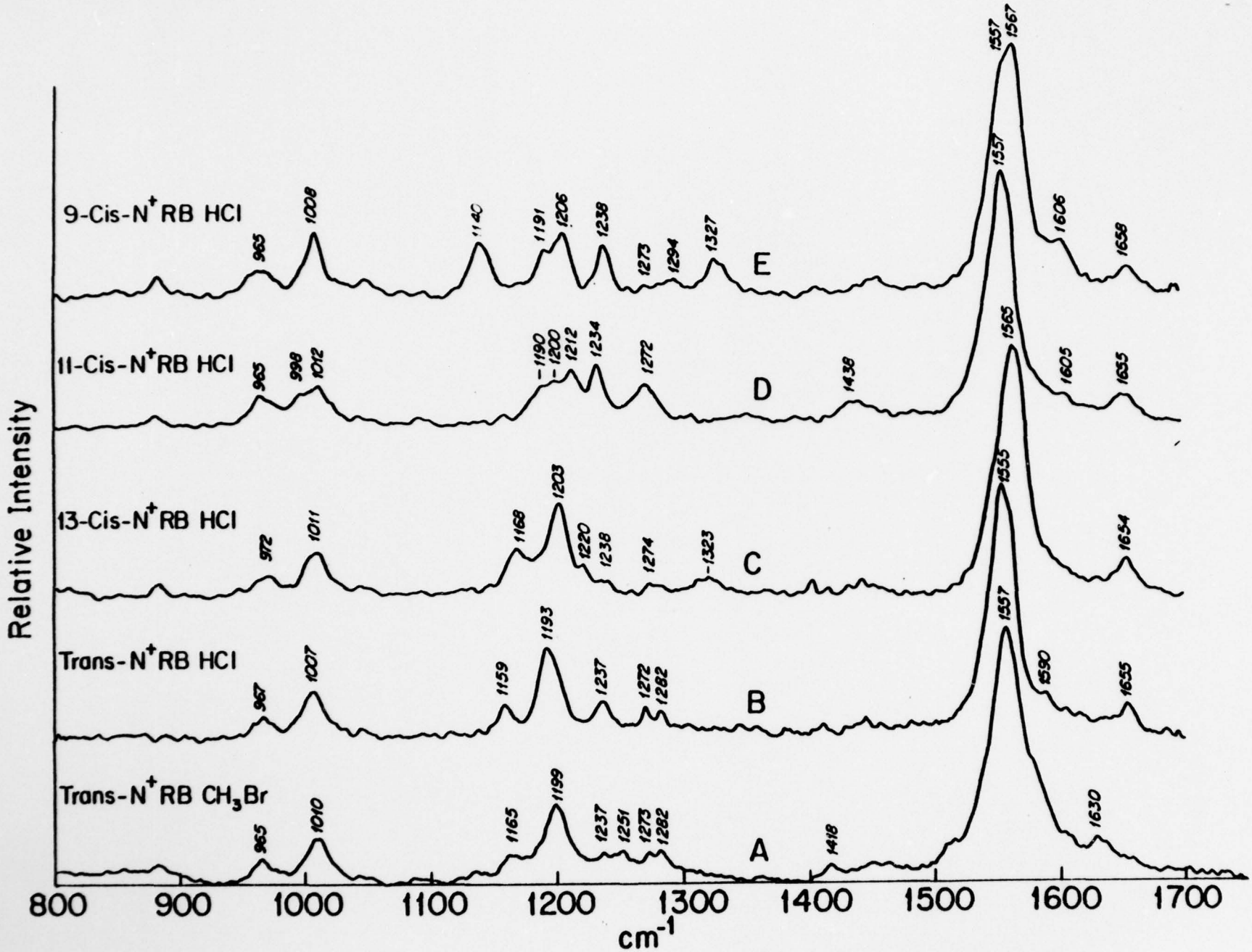

11-cis-Retinal
IR
868
877
894
966
985
1018
1048
1125
1140
1188
1203
1255
1268
1338
1358
1375
1382
1446
1574
1660
Raman
822
970
997
1017
1128
1143
1206
1219
1271
1345
1576
1658
800
1000
1200
1400
1600
Frequency (cm-1)

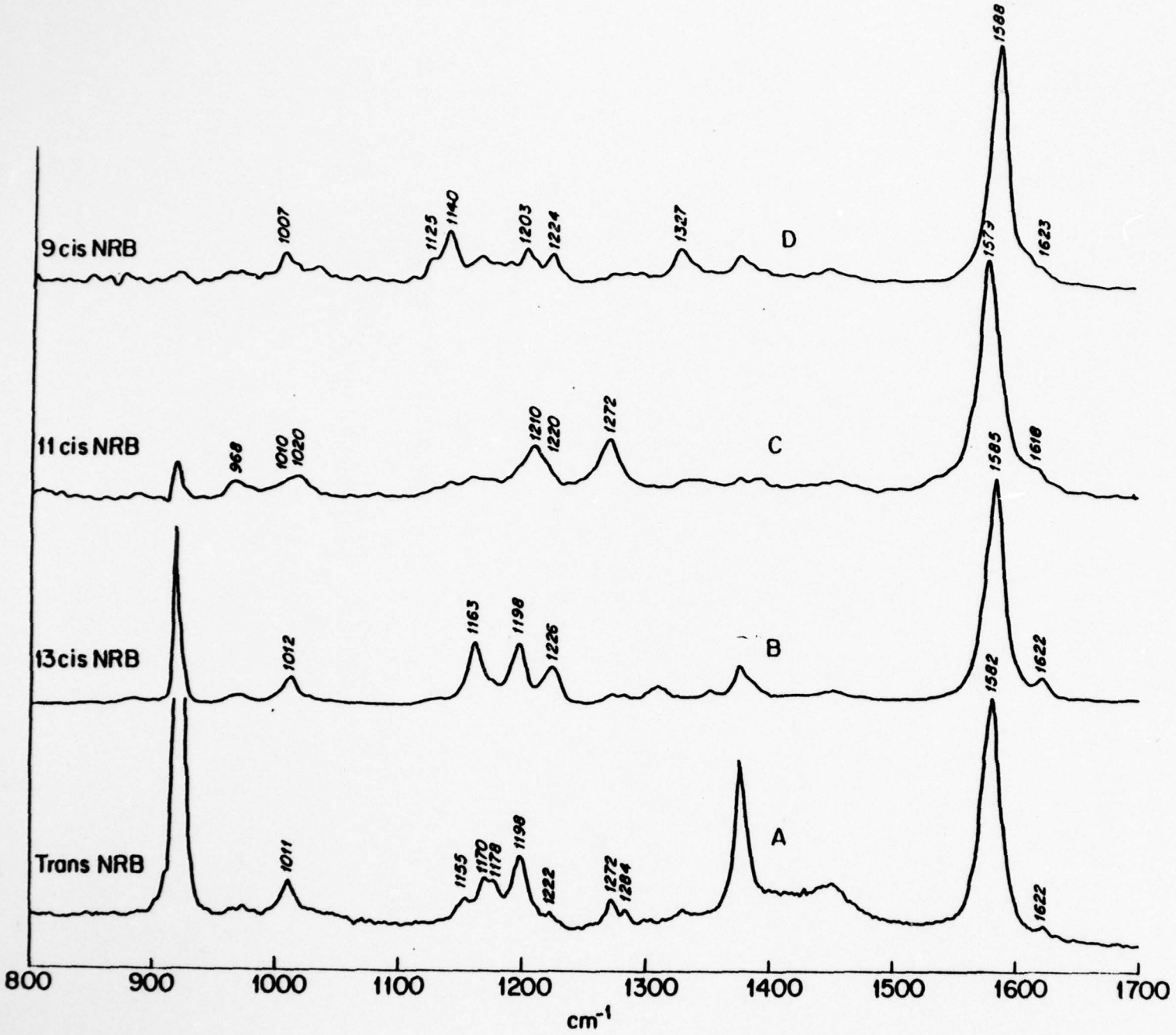

9-cis-Retinal
IR
818
883
963
1026
1114
1148
1184
1200
1217
1256
1274
1289
1334
1360
1379
1401
1446
1582
1658
Raman
963
1009
1031
1117
1147
1187
1201
1216
1280
1295
1329
1337
1586
1656
800
1000
1200
1400
1600
Frequency (cm⁻¹)

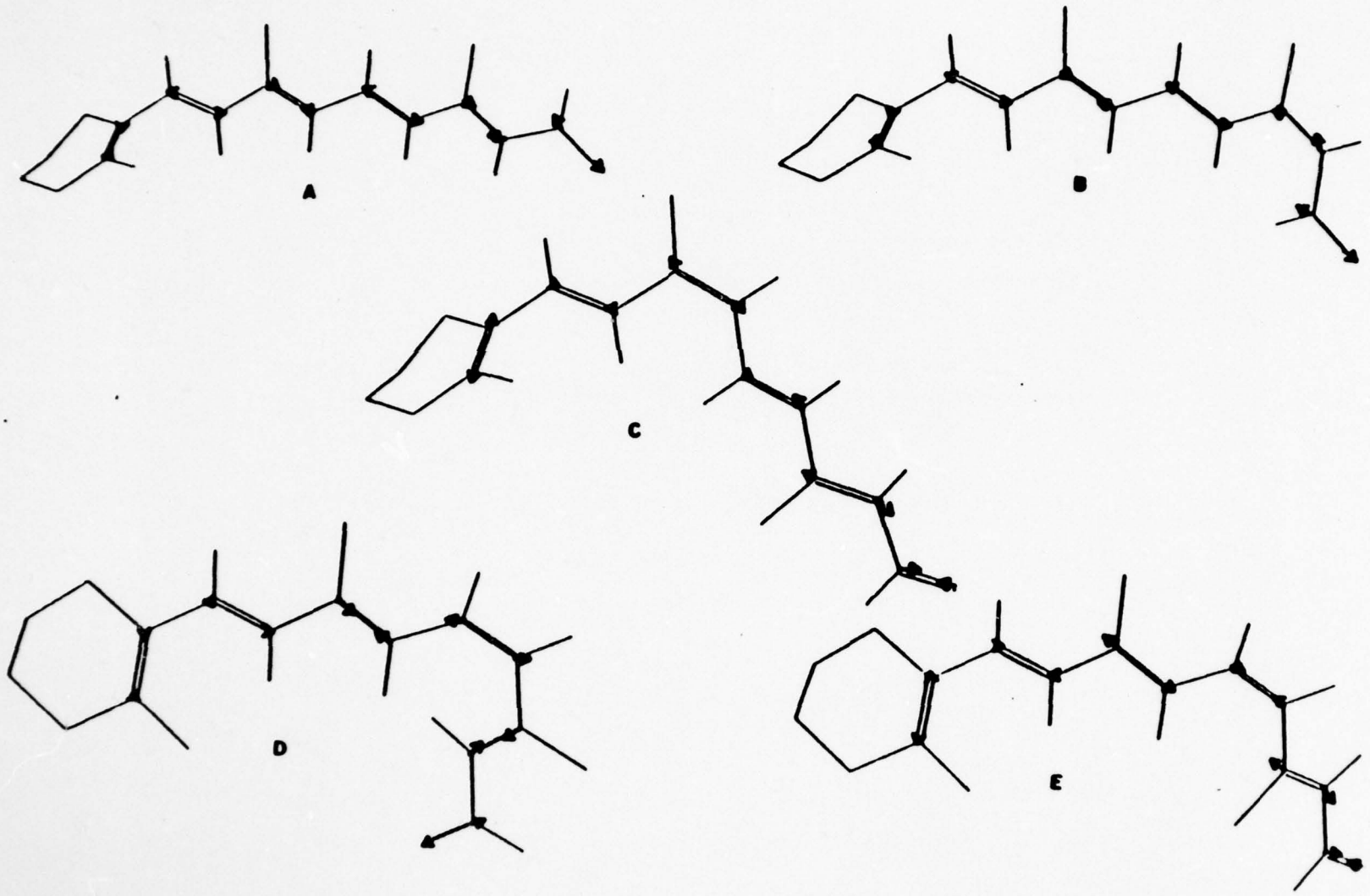

Trans-3-dehydroretinal
IR
876
886
964
1001
1023
1046
1110
1139
1161
1172
1196
1214
1274
1333
1360
1385
1402
1445
1542
1562
1576
1660
Raman
807
852
868
964
976
1009
1162
1174
1200
1277
1337
1564
1578
1657
800
1000
1200
1400
1600
Frequency (cm-1)

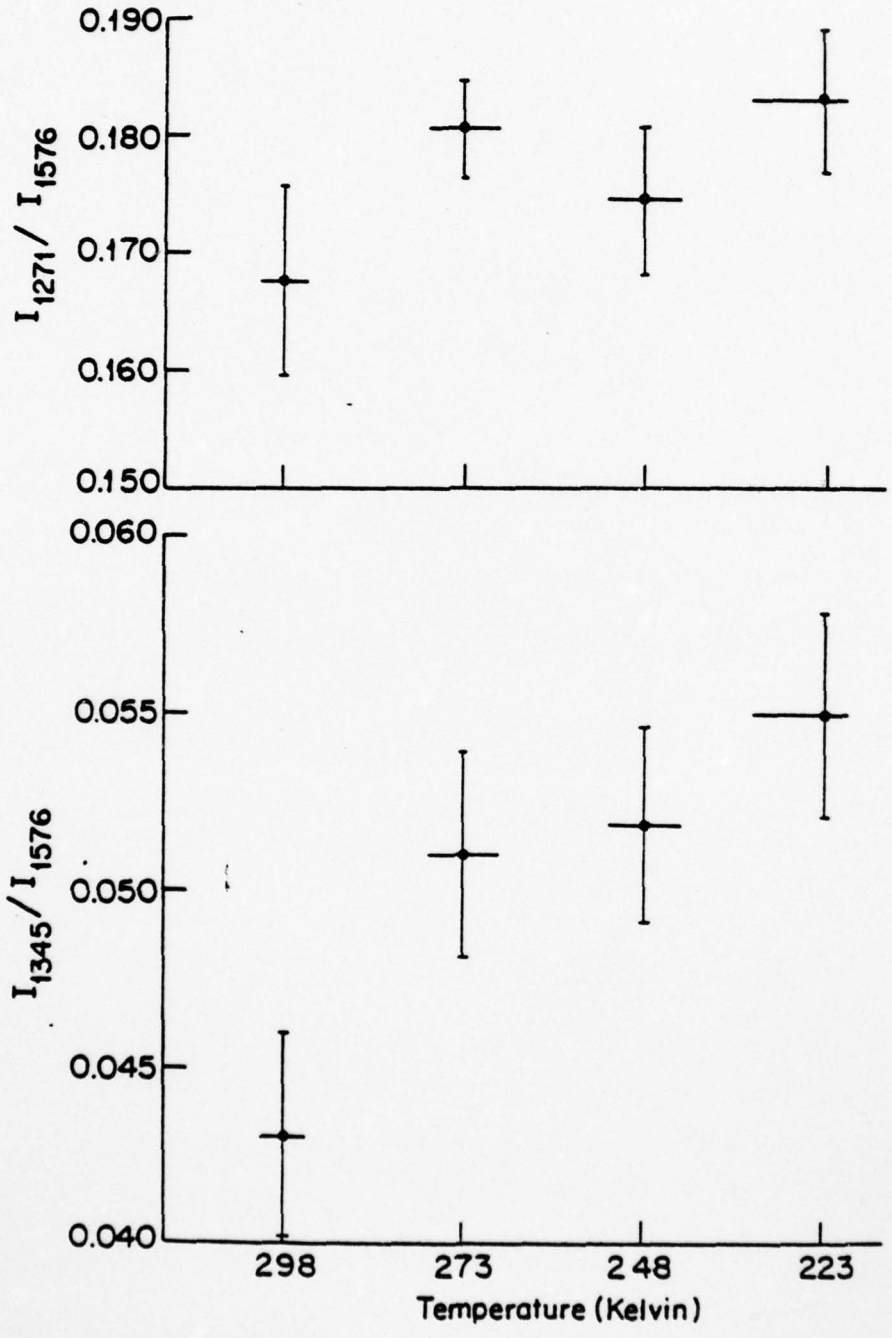

Relative Intensity
Crotonaldehyde
780
932
991
1010
1043
1079
1150
1233
1256
1305
1354
1376
1393
1445
1628
1642
1684
1695
B
β-Ionone
790
816
892
930
939
966
980
1000
1044
1065
1123
1139
1173
1188
1207
1212
1254
1271
1290
1301
1326
1346
1359
1376
1383
1426
1444
1459
1471
1587
1606
1632
1668
1692
A
800
900
1000
1100
1200
1300
1400
1500
1600
1700
cm-1

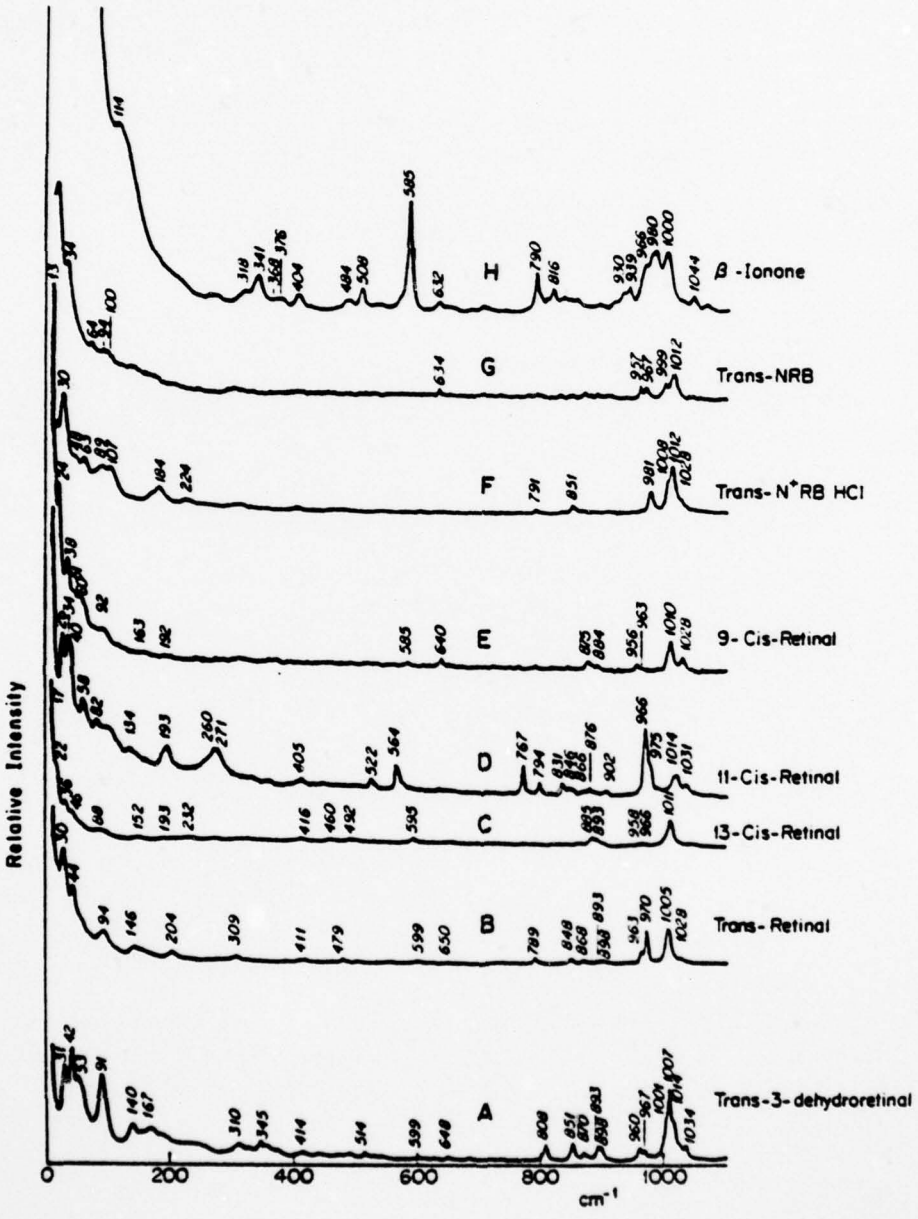

Relative Intensity
9-Cis-Deut, Retinal
11-Cis-Deut, Retinal
13-Cis-Deut, Retinal
Trans-Deut, Retinal
D
C
B
A
617
832
953
966
977
1302
1530
1534
1540
1637
838
895
979
1002
1231
1302
1527
1536
1638
766
810
836
912
955
988
1290
1302
1517
1528
1541
1641
823
902
947
960
980
1304
1638
800
900
1000
1100
1200
1300
1400
1500
1600
1700
cm⁻¹

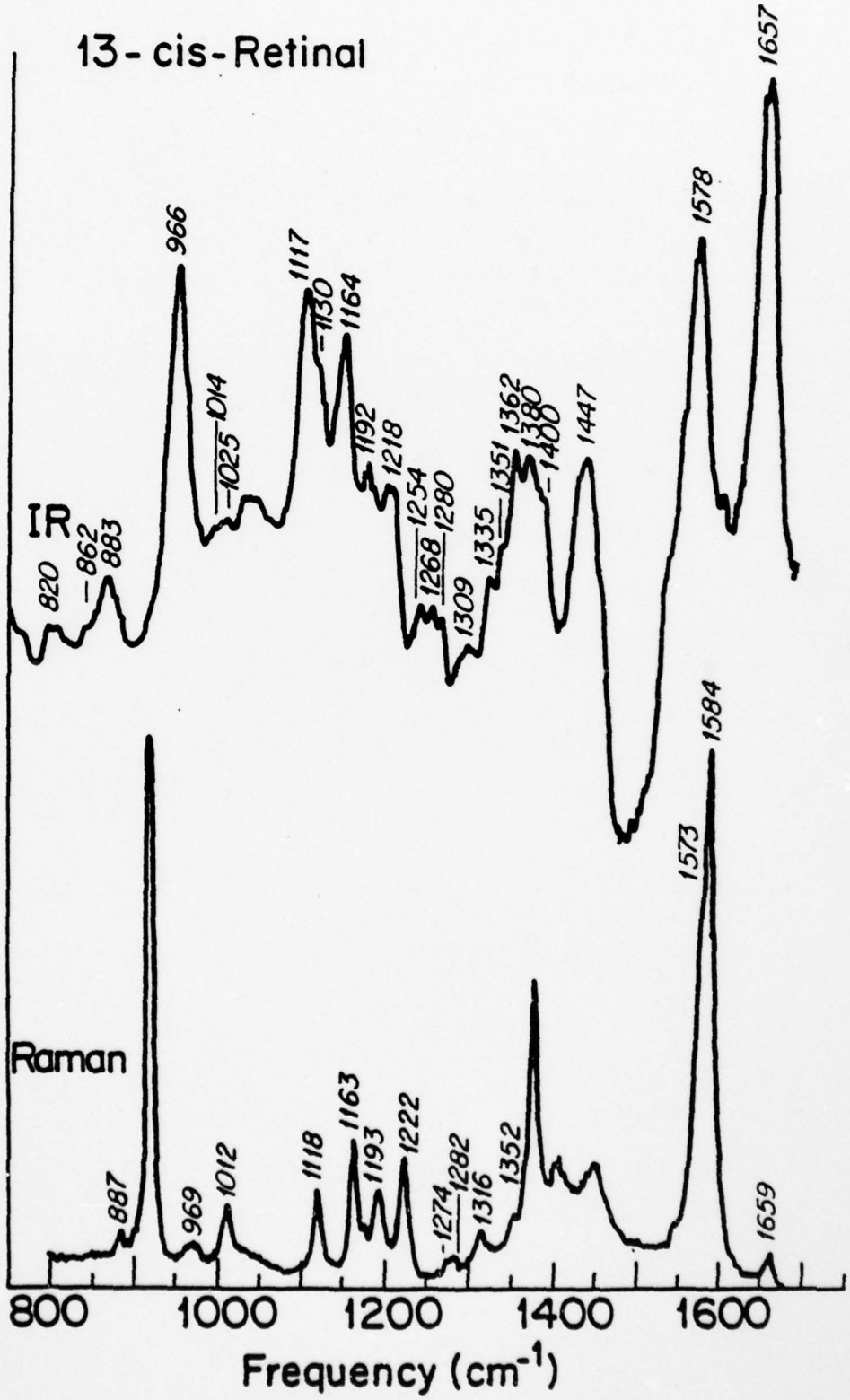

9-Cis-N⁺RB HCl
965
1008
1140
1191
1206
1238
1273
1294
1327
1557
1567
1606
1658
E
11-Cis-N⁺RB HCl
965
998
1012
1190
1200
1212
1234
1272
1438
1557
1565
1605
1635
D
13-Cis-N⁺RB HCl
972
1011
1168
1203
1220
1238
1274
1323
1535
1654
C
Trans-N⁺RB HCl
967
1007
1159
1193
1237
1272
1282
1557
1590
1655
B
Trans-N⁺RB CH₃Br
965
1010
1165
1199
1237
1251
1273
1282
1418
1557
1630
A
Relative Intensity
800
900
1000
1100
1200
1300
1400
1500
1600
1700
cm⁻¹


9 cis NRB
1007
1125
1140
1203
1224
1327
D
1588
1573
1623
11 cis NRB
968
1010
1020
1210
1220
1272
C
1585
1618
13 cis NRB
1012
1163
1198
1226
B
1582
1622
Trans NRB
1011
1155
1170
1178
1198
1222
1272
1284
A
1622
800
900
1000
1100
1200
1300
1400
1500
1600
1700
cm-1


A
B
C
D
E


0.190
0.180
0.170
0.160
0.150
I_{1271}/I_{1576}
0.060
0.055
0.050
0.045
0.040
I_{1345}/I_{1576}
298
273
248
223
Temperature (Kelvin)


Relative Intensity
β -Ionone
Trans-NRB
Trans-N⁺RB HCl
9-Cis-Retinal
11-Cis-Retinal
13-Cis-Retinal
Trans-Retinal
Trans-3-dehydroretinal
cm⁻¹
0
200
400
600
800
1000


13-cis-Retinal
IR
820
862
883
966
1014
1025
1117
1130
1164
1192
1218
1254
1268
1280
1309
1335
1351
1362
1380
1400
1447
1578
1657
Raman
887
969
1012
1118
1163
1193
1222
1274
1282
1316
1352
1573
1584
1659
800
1000
1200
1400
1600
Frequency (cm⁻¹)